ACTA PHYSIOLOGICA SCANDINAVICA

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ACTA PHYSIOL. SCAND

Vol. 68. INDEX

Fasc 1 (September 1966)

Fasc 2 (October 1966)

Fasc. 3—4. (November—December 1966.)

INDEX AUCTORUM

Acta physiol scand 1966 68 1—17

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Active State Durations of Rat Gastrocnemius Muscle

By

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Received 2 August 1965

Abstract

Wilander B *Active state durations of rat gastrocnemius muscle* Acta physiol scand 1966 *68* 1—17

For rat gastrocnemius muscle it has been found that 1 The shortening velocity of an isotonic afterloaded contraction increases with the stimulation frequency up to a frequency of 4[illegible]3 imp/sec

stimulation with frequencies somewhat below that necessary for fusion it has been found that the mechanical latency is as short as after the first impulse (0 6 msec) The maximal rate of tension rise after an impulse is however considerably delayed and the implications of this finding are discussed in terms of the active state concept

During an investigation of the properties of rat gastrocnemius muscle I found that the optimal stimulation frequency for an isotonic contraction against a small load was considerably higher than the lowest frequency giving maximal plateau tension during an isometric tetanus A research program was then started to investigate the fundamental cause of this difference in response to stimulation frequency Such a study is thought to be of some importance for research in muscle physiology as it is apparent that formerly the situation was often thought to be under experimental control when a fixed stimulation frequency was used throughout the contractions During the course of this investigation a paper by Buller and Lewis appeared in which a similar difference in optimal stimulation frequency was described for rate of tension development and for peak tension during indirect stimulation of cat muscle (Buller and Lewis 1965)

According to Hill there exists for a period of time after an impulse a constant level of maximal intensity of the active state which is abruptly developed at the end of the latent period (Hill 1949 1953) In the present paper a distinction will be

drawn between an active state for shortening, which is indicated by the capacity of the muscle to reduce its length and an active state for tension maintenance, which is indicated by the capacity of the muscle to resist stretch, as suggested by Sandow (1960)

Sensitive determinations of the active state plateau duration have been made by superimposing contraction curves for one and two impulses on each other (Macpherson and Wilkie 1954) According to Ritchie, the duration of the active state plateau may also be determined from the time from the last impulse of a tetanic train to the beginning of relaxation after a correction for mechanical latency (Ritchie 1954 *b*) A method related to the latter is the determination of the fusion frequency (Mauriello and Sandow 1953, Ritchie 1954 *b*)

In several papers the duration of the active state plateau after a single impulse is reported to be of the same order of size as the duration of the active state plateau during a fully developed isometric tetanus (Ritchie 1954 *a*, Macpherson and Wilkie 1954, Bleckmann *et al* 1963, Mårtenson and Skoglund 1964) The lastmentioned authors, however, express some doubt about the reliability of determinations of the duration of the active state plateau after a single impulse

The present work will show that there is a considerable difference between a well marked active state plateau for shortening after the first impulse and what is generally considered as an active state plateau for tension maintenance when peak isometric tetanic tension has developed, the first active state plateau being very short The validity of the active state concept will be discussed in relation to the present findings

Methods

were covered with vaseline care being taken not to disturb the contractions with any extra damping The femur was rigidly clamped in a horizontal position in a screw vice One of the clamps passed

ed several times through the remaining soft tissues of the heel as a cathode and the bone clamp as a ground lead, the pulse duration being 0 25 or 0 30 msec

Isometric contractions were recorded by means of a RCA 5734 mechano-electric transducer, the anode pin of which was mounted on the middle of a short brass bar fixed at both ends The muscle was connected with this bar by means of a chain and a light calcaneus clamp The bar was also provided with a light damping pot with a fixed vane The static response of the system was found to be linear, even for very small tensions The natural frequency of the system, with chain

shift and amplitude distortion due to resistances and stray capacitances of the electric system were found to be negligible, compared with those of the mechanical, the rise time for a step change being 0 03 msec The stationary state phase lag of the system for periodic sinusoidal driving functions was calculated to be no more than 0 12 msec up to the natural undamped frequency the apparent latency of a transient response being much less The output of the isometric recording system was fed to a Tetronix 561 oscilloscope with a 72 dual trace amplifier, either directly or through high pass

12 g and the stiffness of the lever corresponded to 60 kp cm at the muscle end

For the recording of action potentials a Tetronix 122 preamplifier was used and the bipolar electrode consisted of 0 2 mm twisted copper wire, which was insulated except at the tips which were introduced into the distal end of the muscle

Results

A comparison between the dependences upon stimulation frequency of isometric peak tension and isotonic shortening velocity

The isometric contractions were recorded at the muscle length at which resting tension just disappears At this length the tetanic tension soon reached a constant mean value, even at rather low stimulation frequencies The tension was measured at 150 msec of stimulation For unfused tetani the tension was measured to the peak of the oscillations Apparent fusion seemed to occur at about 200—225 imp sec at the amplification used in the tension determinations The isotonic contractions were afterloaded and started at the optimum length for twitch peak tension against a load of 100 pond (A comparison on the active state plateau durations at different lengths is presented in the third section of the results The shortening velocities were measured after about 10 msec of stimulation, when the velocity became fairly constant At the lowest frequency slight oscillations sometimes occurred the velocity was then measured over the peaks of these oscillation The stimulation intensity was supramaximal, even for high frequency stimulation For a discussion of the proper conditions for direct stimulation, see the next section The recordings were made in an ascending or descending order of frequencies Every third contraction was a reference contraction at 180 imp sec for isometric and 400 imp sec for isotonic contractions Interpolated corrections were performed to compensate for slight

TABLE I A Isometric tetanic tension at 150 msec at different stimulation frequencies

Stimulation frequency imp/sec		130		160		180		200	
Direct stimulation	Relative tension	96 1		98 2		100 0		100 9	
	S E		0 61		0 55		0 31		0 26
	p		0 012		0 015		0 022		0 058
Indirect stimulation	Relative tension	96 3		99 0		100 0		101 1	
	S E		0 57		0 17		0 27		0 23
	p		0 006		0 002		0 01		0 7

Tensions and velocities are presented in relation to the reference contractions at 180 and 400/sec the velocities for indirect stimulation being presented in relation to the velocity for direct stimulation at 400/sec S E =standard error of the mean of the relative differences in animals between the adjacent stimulation frequencies p calculated risk of making a wrong decision when rejecting the

TABLE I B Isotonic shortening velocity at different stimulation frequencies

Stimulation frequency imp/sec		180		300		375	
Direct stimulation	*Relative velocity*	*79 8*		*90 7*		*96 8*	
	S E		1 26		0 17		1 36
	p		*0 001*		*0 000*		*0 039*
Indirect stimulation	Relative velocity	73 1		82 1		85 0	

progressive changes in response The largest total correction varied from 1 % to 6 % between the different preparations

The depence of isometric tension and of isotonic shortening velocity upon stimulation frequency were investigated both for direct and for indirect stimulation The results are shown in Table I Statistical calculations according to the common t test were performed on the relative differences of responses in animals between adjacent stimulation frequencies Some calculations for larger frequency intervals are also shown The deviates for the frequency intervals between 250 and 180 imp/sec, isometric registration were pooled and found to be approximately normally distributed, as small differences in similar animals often are The tests were one tailed, as the possible direction of change is well known

For isometric contraction the results are quite in agreement with the often-quoted conclusion of Cooper and Eccles (1930), that above the apparent fusion frequency no substantial *increase in tension occurs* *In* this case the tension increases signifi

225		250		275		300		400	
									300—225
101 4		101 5		101 5		101 9		99 8	
	0 32		0 68		0 43		0 53		0 33
	0 3		—		0 17		0 008		0 3
									275—200
101 2		101 3		101 7		101 3		99 8	
	0 24		0 49		0 39		0 86		0 42
	0 7		0 5		0 2		0 08		0 2

null hypothesis that no difference exists in favour of the hypothesis that a difference exists in an expected direction In the last column to the right analyses of larger frequency intervals are presented Number of animals I A direct stimulation, data pooled from 7 rats, 5 differences for each frequency interval *I A indirect stimulation, 6 rats I B, 5 rats on which records for indirect as well as direct* stimulation were obtained

400		425		450		500	
							425—375
100 0		*102 7*		*100 6*		*101 6*	
	0 83		1 36		0 81		1 49
	0 019		0 2		0 2		0 008
86 9		*67 4*		*87 8*		*89 6*	

cantly up to about 200 imp/sec for indirect and for direct stimulation and then seems to remain on a plateau within the limits of accuracy of a small number of experiments At 400 imp/sec a decline due to some refractoriness is evident In isotonic contractions during direct stimulation, however, the response increases significantly up to about 425 imp/sec, a frequency about twice as high as the corresponding frequency for isometric tension at 150 msec

No statistical calculations are presented for the indirect isotonic data, the course of the velocity-frequency curves being rather irregular The highest maximum is often not obtained until 500 imp/sec (cf Buller and Lewis 1965 The velocities by indirect stimulation were always lower than those by direct stimulation and it is obvious that maximal shortening velocity is not attained by indirect stimulation Such a difference was not evident for isometric contractions, maximal response being obtained at lower frequencies

TABLE II A. Results obtained from isometric contraction records for one and two impulses

Direct stimulation										Indirect stimulation	
Column 1	2	3	4					5	6	7	8
Animal number	Period of latency	L. R. start to tension minimum	L. R. start to separation. Stimulation intervals					Shortest separation time	Stimulation interval for 5	Shortest separation time	Stimulation interval for 7
			0.8	0.9	1.0	1.1	1.2				
1	0.65	0.33	1.85	*1.44*	*1.42*	*1.44*	—	1.42	1.0	1.66	1.0
2	0.65	0.30	1.66	*1.36*	*1.36*	1.41	1.47	1.36	0.9 1.0	1.71	1.0
3	0.54	*0.49*	1.78	*1.59*	*1.55*	*1.66*	*1.71*	1.55	*1.0*	*1.72*	*1.1*
4	0.60	0.49	*1.37*	*1.36*	*1.39*	1.52	1.66	1.36	0.9	1.69	1.0
5	0.54	0.60	*1.58*	*1.58*	1.71	1.85	—	1.58	0.8 0.9	1.63	1.0
6	0.65	0.36	1.83	*1.45*	*1.45*	1.49	1.61	1.45	0.9 1.0	1.70	1.0
Mean ± S.E.	0.61 ± 0.02							1.45 ± 0.04		1.69 ± 0.014	

Shortest time from tension minimum during latency relaxation to separation 1.03 ± 0.04

Difference between shortest separation time at indirect stimulation and at direct stimulation 0.23 ± 0.05

All time values in milliseconds

Explanations

1. The animals are the same as those in table II B
2. The latency is measured from the start of the stimulating pulse to the earliest sign of latency relaxation
3. The time is measured from the earliest sign of latency relaxation to the lowest point of the curve
4. The times are measured from the earliest sign of latency relaxation to the point where the contraction curves for one impulse and for two impulses at the indicated intervals separate

The italics show values for each animal which do not differ by more than 0.03 msec, i.e. the accuracy of separate readings, the represented values being means of one run of increasing and one run of decreasing stimulation intervals

An analysis of the active state plateau duration after a single impulse

According to the method of Macpherson and Wilkie, the duration of the active state plateau after a single impulse can be determined by superimposing a contraction curve, in which the active state is prolonged at maximal level by a second impulse given early enough on a twitch contraction curve (Machpherson and Wilkie 1954, Norris 1961). The point where the contraction curves separate then indicates the end of the first active state plateau. According to Hill (1949), the contractile elements shorten against the series elastic elements during the ascent of an

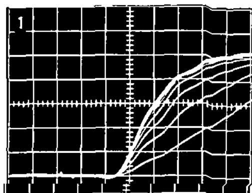
isometric contraction, and it will be considered here that the earliest obtainable separation point indicates the end of the active state plateau for shortening

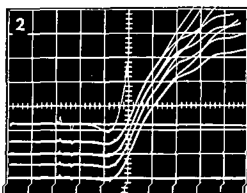
The isometric contractions were recorded at high sensitivity, about 3/1000 of the peak tetanic tension per cm of the oscilloscope screen To keep a fixed base line and to improve the resolution of the separation, the oscilloscope was connected via an RC high pass filter with a time constant of 1 msec The contractions were recorded at the optimum muscle length for twitch peak tension

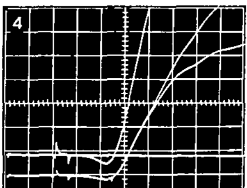
As shown by Macpherson and Wilkie, the estimate is dependent upon the synchronism of activation To get a synchronous activation, the starts of the twitches were recorded at the same amplification, sweep speed and filter time constants as was used for separation studies The stimulation intensity was increased in steps and the start of the twitches were recorded for several steps on the same film (Fig 1) The rate of tension development first increased for each step and the latency relaxation and the special form of the early phase of the contraction curve to be described later appeared more distinctly After a certain stimulation intensity, determined for each preparation, the starts of the twitches became perfectly superimposed This stimulation intensity was appreciably higher than that just supramaximal for twitch peak tension The stimulation intensity used was somewhat higher than that just supramaximal for rate of tension rise

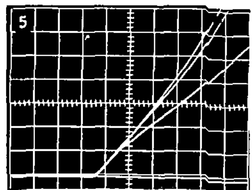
In order to obtain a reliable measure of the active state plateau duration it is necessary to avoid repetitive firing for the first pulse The absence of repetitive firing is indicated not only by the short plateau duration obtained but also by the constancy of twitch peak tensions (cf Ritchie 1954 *a*) for stimulation intensities from 20 to 30 V below to 20—30 V above the stimulation intensity used for synchronous activation (Range 150—170 V over the electrodes, 8 animals) To avoid repetitive firing, the pulse duration must be short (Gjone 1955) It is also necessary to prevent the high field intensity in the neighbourhood of a wire electrode in a volume conductor from reaching the muscle by placing the electrodes at some distance from it

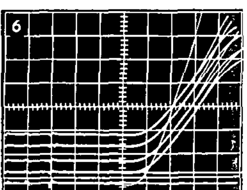
Table II A col 4, gives for the separate animals the time intervals between the beginning of latency relaxation and the separation point of the contraction curves for direct single pulses and two pulses at different stimulation intervals The values represent the means of one run of increasing and one run of decreasing stimulation intervals A representative run is shown in Fig 2 The italics in Table II A represent the time values which do not differ by more than the accuracy of 0 03 msec with which the readings were performed If there is a plateau of active state initiated by the first impulse and if the second impulse when it comes early enough only prolongs the duration of this plateau, then there is a possibility that the time between the beginning of the latency relaxation and the separation point is constant for a range of stimulation intervals As is evident from the results it has been possible to obtain such constancy in the experiments in spite of the difficulty of obtaining a full response for a second pulse at such short intervals An interesing effect is always obtained at stimulation intervals shorter than 0 8 msec, where the contraction curve for paired stimuli first goes below the curve of the single stimulus, as shown in Fig 3











[illegible] on intensities
[illegible]lt steps up to
[illegible]eak isometric

[illegible]li The upper
[illegible]paired stimuli
[illegible]

Whatever the cause of this inhibiting effect on the first active state of an early second pulse, the effect shows that it is not possible to obtain shorter estimates of the plateau duration by this method A similar effect was obtained by Brady, Abbott and Mommaerts (1961) in cardiac muscle for a train of pulses during the absolutely refractory period

It may be further questioned whether there is a sufficiently long plateau of the active state, if any, or if the shortest time obtainable between the start of the first pulse and the separation point is limited by the sum of the irresponsive period and a mechanical latency for the second action potential (cf Mårtenson and Skoglund 1964) As shown in Fig 2 and 4, the second impulse causes a prolongation of the initial course of the contractions if the separation occurs early enough It is the contraction curve of a single impulse which deviates from the initial course after the separation The same effect is also shown without filter distortion, with the oscilloscope DC-coupled, in Fig 5 Here also contraction curves for two and three impulses is shown, where the same effect occurs once more, although it is less marked In spite of a slowly declining active state, the later positive acceleration may possibly be largely ascribed to an increasing stiffness of the series elastic elements (cf Sandow 1960) At no tension after the separation is the rate of tension development of the twitch as high as that of the contraction induced by two stimuli This is not in accordance with the results of Close (1962)

One cannot escape the impression that the form of the contraction curves for one, two and three direct pulses shown here are consistent with the view that soon after a stimulating pulse there is a period of high and possibly approximately level activity which declines long before the twitch peak tension is reached, and that repeated stimulation can do no more than cause a prolongation of the initial high level of activity It cannot be said, however, that this activity is exactly as high as that during a fully developed tetanus This result has been obtained without any imposed disturbance of the muscle, as in Hill s experiments (1949, 1953) the interpretation of which has been criticized on this account by several authors (Goodall 1957 Pringle 1960 Walker 1960, cf also Sandow 1960)

The obtained form of the contraction curves is probably not only due to the fidelity of the isometric recording system and the synchronous activation but also to small variations in properties in a dominant group of fibres

The interpretation of the recorded form of the early phase of an isometric twitch presented here has to be compared with that of Sandow (1958 1960 who suggests that the active state hump on his differentiated records indicates the termination of the development of the active state

Fig 5 Direct stimulation Superimposed isometric contraction curves for one impulse and for two and three impulses at intervals of 0 9 msec Time 1 msec div Ordinate about 1 5 ° of maximal isome... Oscilloscope DC-coupled
isometr contraction curves for single and paired 0 9 and 0 8 msec The lowest curve shows the start he stimulation artifacts there are triggering artifacts

So much may be said about the end of the first active state plateau for shortening but when does it begin? In Fig 2 and 4 the start of an isometric twitch is shown at about 2 5 × greater amplification than that used for separation studies The time after an impulse at which the latency relaxation begins is not influenced by the muscle length, as long as there is some resting tension, so that a drop in tension is evident (Sandow 1944 Abbott and Ritchie 1951) Hill (1951) has shown that an increase in the resistance to stretch occurs in the stimulated muscle, evidently at about the same moment as that at which the latency relaxation begins, and before there is any evidency of shortening The time from the start of the stimulating pulse to the first evidence of relaxation will be arbitrarily described here as a period of mechanical latency, a period during which there is no sign of activity in the records The values shown in Table II A, col 2, are the shortest hitherto reported

The values reported in Table II A, col 5, for the time interval between the beginning of relaxation and the earliest obtainable separation cannot be regarded as reliable measurements of the plateau duration but they represent the largest possible values of the plateau duration after a single impulse about 1 5 msec As is evident from recordings like Fig 5 where also the duration of the second active state plateau may be estimated, the earliest active state plateaus are probably not much longer than about 1 msec

The longest possible value for the duration of the active state plateau after a single impulse about 1 5 msec is considerably shorter than earlier estimates, even or mammalian muscles The value reported by Norris (1961) for rat peroneus digiti quinti is about twice as long

With indirect stimulation as is evident from the results in Table II A col 7 the shortest obtainable times from the beginning of latency relaxation to the separation point are somewhat longer than those with direct stimulation There are also no clear indications of any active state plateau in the records A representative run is shown in Fig 6 The asynchronous activation and the duration of the irresponsive period obviously interferes with the active state determinations in this case these effects however being slight for this muscle

An analysis of the duration of the active state plateau during a fully developed isometric tetanus

According to the method of Ritchie (1954 *b*) the duration of the active state plateau may be estimated by determining the time between the last impulse during a just fused tension plateau and the beginning of relaxation after a correction for the period of mechanical latency A similar estimate would be obtained from the stimulation interval just necessary for fusion if this could be determined exactly enough

Determinations of this kind were made with the oscilloscope sweep triggered by one of the last impulses of a 150 msec pulse train of various frequencies The oscilloscope was coupled through an RC high pass filter with a time constant of 10 msec which permitted recording with a sensitivity of about 1/1 000 of maximal isometric tetanic tension (about 5 kp per cm of the oscilloscope screen without distortion of the rapid events studied The sweep speed was 1 or 2 msec/div As the stimulation

TABLE II B Results obtained from isometric contraction records at 150 msec of direct tetanic stimulation

Column 1	2								3	4	5
Animal number	Time from the start of the last stimulating pulse to relaxation at stimulation frequency								Empirical fusion frequency	Time from the last impulse at fus freq to start of relaxation	Time from the last impulse at fus freq to 1/1000 tension fall
	175	200	250	275	300	325	350	400			
1	5 4	5 9	3 9	3 7	4 0	3 7	—	—	300	4 0	6 3
2	5 8	4 8	3 9	4 1	3 5	3 7	3 6	3 0	325	3 7	6 1
3	4 7	—	—	—	3 3	3 4	3 4	3 4	350	3 4	6 9
4	4 4	4 6	4 6	4 1	4 1	4 2	4 1	4 4	300	4 1	7 0
6	4 8	4 8	4 4	4 2	4 2	4 0	4 2	2 4	300	4 2	6 4
Mean ± S E										3 9 ± 0 15	6 5 ± 0 17

Column 6	7	8	9	10	11
Animal number	Time from an impulse to max rate of tension rise at 175 imp/sec	Difference between col 7 and col 4	Difference between col 4 and initial mech latency (Table II A)	Col 9 divided by the shortest sep time (Table II A)	Calculated fusion frequency according to col 9
1	2 7	1 3	3 3	2 3	300
2	2 5	1 2	3 0	2 2	330
3	3 0	0 4	2 9	2 0	340
4	3 0	1 1	3 5	2 6	290
6	2 6	1 6	3 5	2 5	290
Mean ± S E	2 8 ± 0 10	1 1 ± 0 20	3 2 ± 0 12	2 3 ± 0 12	310 ± 10

Time values in milliseconds Frequencies in impulses sec

artifact proved to give a convenient and exact measure of the time of the start of a pulse without chopping, it was not reduced Measurements were performed at the length at which resting tension just disappears and a few determinations at the optimum muscle length for twitch peak tension a difference of about 5 mm are also reported

The measurements performed are indicated in Fig 8 the results are given in Table II B and a representative record of some of the measurements in Fig 7 The

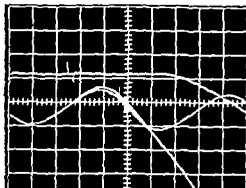


Fig 7 Direct stimulation Below Isometric oscillations obtained at 175 imp/sec for 150 msec The sweep is triggered via a relay by the last pulse in one case and by the last but one in the other case Latency is measured from the stimulation artifact to the time when the forms of the curves deviate from each other the records being read with a magnification of 5 × As the curves are [illegible] sec Sweep triggered by the last pulse but one The fusion is almost complete Filter time constant 10 msec *Time 1 msec/div Ordinate 1/1000 of* isom max tension (about 5 kp)/div

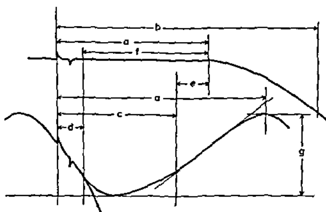


Fig 8

Above The events after the last impulse of a train at fusion frequency
Below Oscillations during a not fully fused isometric tetanus with a relaxation curve after the last impulse superimposed
The following measurements have been performed
(*a*) The time from the start of an impulse to the beginning of relaxation
(*b*) The time from the start of the last impulse to 1/1000 fall of tension
(*c*) The time from the start of an impulse to the maximal rate of tension rise
(*d*) Determination of the latent period
(*g*) Oscillation amplitude
(*e*) and (*f*) have been calculated from the above determinations

stimulation was direct The time intervals measured did not vary by more than a very small percentage in the course of an experiment and were also approximately constant for an interval of stimulation intensity between the intensity which was used (180 to 200 V) and an intensity 30 V less

Around the fusion frequency determinations were made in steps of 25 imp/sec The frequency above that at which faint oscillations were still visible was arbitrarily described as the empirical fusion frequency (col 3) The time between the start of an impulse and the following peak or for frequencies above fusion frequency, to the

point where the curve began to deviate from the preceding level course, did not vary much around the empirical fusion frequency (col 2) According to Hill (1949), the recorded tension at the peaks, as well as during the level course, is just equal to the tension, P_0, the contractile elements are able to bear when neither shortening nor lengthening For frequencies below fusion, P_t is declining at the peaks and the times in col 2 are generally longer the lower the frequency A slight shortening of the times from pulse to peak is sometimes apparent at intermediate frequencies This effect, also described by Cooper and Eccles (1930), is more marked at frequencies around 100 imp/sec, where a transient deepening of the oscillations occurs These effects will be further described and analysed in a later paper For frequencies above fusion, the last impulse might sometimes occur too early to cause a prolongation of the plateau for as long a time as that for lower frequencies For frequencies higher than that for empirical fusion, a short and slight inhibiting effect after each pulse may be seen, an effect resembling that described in the preceding section In order to obtain a good estimate of the duration of the active state plateau by Ritchie's method, it is necessary to select the right frequency

In four experiments the muscle was stretched about 5 mm to the twitch optimum length The determination of the time between the last pulse and the beginning of relaxation at the empirical fusion frequency then gave, in relation to that obtained at the shorter length, no change in one experiment and a prolongation of 0 1 msec in three experiments This slight effect on the time to the estimated termination of the active state plateau may be an apparent one, caused by a retarding effect of the parallel elastic elements No effects were evident on the form of the oscillations at lower frequencies

The values for the time intervals from the last impulse at fusion frequency to relaxation (col 4) need a correction for mechanical latency to give an estimate of the duration of the active state plateau In three special experiments a careful comparison was made between the form of the relaxation curve after the last impulse and earlier oscillations at a stimulation of 175 imp/sec (Fig 7) The mechanical latency from the start of an pulse to detectable positive deviation was found to be 0 1 msec longer than the time from the start of the first pulse to the beginning of latency relaxation This difference is equal to the combined stationary state phase shift of the transducer and the high pass filter in the actual frequency range of sine wave components determined from the phase shifts introduced by filters with different time constants There is thus no detectable difference between the inital mechanical latency and the latency after a 150 msec train at 175 imp/sec The mechanical latency during a fused tetanus cannot, of course, be determined directly The correctness of using the initial mechanical latency for the actual correction may, however, be estimated by subtracting the initial latency from the time interval between the start of the last impulse and the beginning of relaxation at the empirical fusion frequency The times calculated in this manner should correspond to the stimulation interval just giving fusion The fusion frequencies calculated in this way show good agreement with the empirical fusion frequencies (cols 11 and 3 respec-

tively) determined with frequency steps of 25 imp/sec The values shown in col 9 with a mean (± the standard error of the mean, S E) of 3 2 ± 0 1 (5 animals) are then to be regarded as estimates of the duration of the plateau of the active state for tension maintenance according to Ritchie The interpretation of these determinations will, however, be reconsidered in the discussion

Two more kinds of measurement were performed on the records, the results of which will be further analysed in the discussion The times from the last impulse at fusion frequency to 1/1,000 fall of tension are shown in col 5 The times from an impulse at 175 imp/sec to the attainment of the maximal rate of tension rise, measured in the way shown in Fig 8 c, are given in col 7 A similar delay in the maximal rate of tension rise is evident for frequencies from 200 imp/sec up to the fusion frequency, although the oscillations cannot be analysed with the same accuracy The time intervals between the attainment of the maximal rate of tension rise at 175 imp/sec and the start of relaxation at fusion frequency are shown in col 8

As the experiments were performed on the same animals, a comparison in animals of the largest possible duration of the plateau of the active state for shortening after a single impulse and the duration of the plateau of the active state for tension maintenance at 150 msec stimulation at fusion frequency can be made The ratios are shown in col 10

No determinations of the kind described in this section were performed during indirect stimulation on these animals In preliminary experiments the oscillations were of lower amplitude and more sine-wave shaped than those with direct stimulation and the records showed signs of repetitive firing after the last impulse A larger hump after the preceding regular oscillations at frequencies below fusion generally occurred

Discussion

The calculated fusion frequency of 310 ± 10 (S E) imp/sec in Table II B, col 11, is appreciably higher than the frequency of about 200 impulses/sec above which no substantial increase in isometric tension occurs The values for the time interval between the last impulse at fusion frequency to 1/1 000 tension fall (Table II B, col 5) correspond, after a correction for mechanical latency, to a stimulation frequency of about 170 imp/sec It has to be realized however, that according to Hill (1949), the contractile elements are being stretched by the series elastic elements during the relaxation of an isometric contraction and that, according to Katz (1939), a muscle is able to resist considerable tensions with only a very slow lengthening Presumably it is largely this great ability of muscle to maintain achieved tension even during the early phase of decline of the active state that enables the muscle to "climb" up to almost maximal tension at 200 imp/sec, in spite of the stimulation interval being nearly 2 msec longer than the fusion interval that can be determined by a highly sensitive method Owing to the steeper course of the force velocity curve for shortening (Katz 1939), we must then expect the shortening velocity to be more

dependent upon stimulation frequency up to fusion frequency than isometric peak tension But the isotonic shortening velocity after 10 msec of stimulation increases with the frequency up to about 425 imp/sec, a frequency appreciably higher than the determined fusion frequency for the isometric tension plateau after 150 msec of stimulation In line with this high optimal frequency is the very brief duration of the plateau of the active state for shortening determined for the twitch no longer than about 1 5 msec The frequency of 425 imp/sec corresponds, however, to a stimulation interval of 2 35 msec There may be some prolongation of the duration of the active state plateau, as shown by Ritchie and Wilkie (1955) for the later stages of the active state after previous stimulation although the effect may be overestimated, owing to a difference in sensitivity of methods and a possible influence of refractoriness

The estimated duration of the plateau of the active state for tension maintenance at 150 msec of stimulation at fusion frequency (Table II B, col 9) of 3 2 ± 0 1 msec is considerable longer than the duration of the plateau of the active state for shortening after a single impulse The maximal rate of tension rise after an impulse at a stimulation frequency somewhat below fusion, however, is not reached until near the end of the active state plateau as estimated by the method of Ritchie The differences are shown in Table II B, col 8 In the tension region in which the oscillations occur, the fully activated muscle has a tension time curve which is convex upwards, as in the ordinary myogram As long as the tension time curve is concave upwards, the muscle is not fully activated The maximal rate of tension rise after an impulse indicates thus the beginning of the active state plateau for shortening (cf Sandow 1960) It is evident that this active state is delayed in relation to that for shortening of a twitch In the further analysis of these results two possible hypotheses that are independent, but do not exclude each other will be advanced

Hypothesis I The active states for shortening and for tension maintenance do not run in parallel This explains why there is such a discrepancy between the duration of the active state plateau estimated by Ritchie s method and the difference between the beginning of the active state plateau for shortening and the end of the active state plateau according to Ritchie s method Further evidence is given by Hill s finding (1951) of a very early increase in resistance to stretch during the latent period which may be compared with the latency estimation at 175 impulses/sec described in the third section of the results Support for this hypothesis is also given by the results of Jewell and Wilkie (1960) which show that during the relaxation of a twitch the tension at which the contractile elements neither shorten nor lengthen declines relatively more rapidly than the maximal velocity of shortening

Hypothesis II The intensity of the active state cannot be increased above that existing during the plateau but the activity caused by one impulse may summate with the activity caused by another impulse up to the limit set by the activity during a plateau The maximal activity caused by one impulse is not developed abruptly and the activity during this rising phase may summate with the declining activity after the preceding impulse When the impulse interval is short enough, fusion may occur, although the duration of the active state plateau for frequencies below fusion

is still shorter than this interval The duration of the active state plateau may even be as short as that of a twitch and the active state plateaus for shortening and for tension maintenance may coincide, although these phenomena are not necessary consequences of the second hypothesis If the plateaus of the two active states coincide, however, Table II B, col 8, gives an estimate of the duration of the active state plateau of 1 1 msec, about as short as that of a twitch, but the plateau is more delayed in relation to the impulse Summation up to a certain limit, of the kind described above, may occur if a plateau of maximal activity is caused by a saturation of the contractile elements with an activating agent, the concentration of which is increased above that for saturation by each impulse and then decreases

A well known explanation of the potentiation of a twitch is a prolongation of the active state From the present work it appears that the rate of development of the active state is variable and this introduces a possible explanation of potentiating effects in which there is an increase in rate of tension rise and a shorter time to peak, as described, for example, for heart muscle (Brady *et al* 1961) although the mechanisms of variation are not yet known The results presented, however, also stress the importance of using a stimulus that is supramaximal for the event studied, e g rate of tension rise, and this must be especially important when the potentiating agent has an effect upon the excitability

A smaller response for indirect stimulation than for direct, as obtained for isotonic shortening velocity, is classically attributed to a partial neuromuscular block It is suggested here that another cause may also be the extinguishing of nerve impulses by collision, as synchronous muscle action potentials may re-excite the intramuscular nerve fibres (Lloyd 1942, Leksell 1945, Brown and Matthews 1960) If the time intervals involved are considered, it appears that possibilities of collision exist at high stimulation frequencies The high optimal frequencies observed for indirect stimulation, which are not consistent with a predominating effect of Wedensky inhibition, may then be explained by more impulses reaching the muscle without collision when the stimulation frequency is increased It should be noted here that with indirect stimulation there is a difference between a short stimulation interval and the first action potential interval in muscle (Eccles and O'Connor 1939) The records showed, however, that later action potential intervals followed the stimulation frequency sufficiently not to influence the interpretation of the responses to indirect stimulation

Even if it is impossible for the nervous system to generate impulses at such short intervals as the duration of the plateau of the active state for shortening after a single impulse, the very short plateau duration may be of functional significance for brief corrections of movements when they tend to deviate from their intended course

The present investigation was supported by grants from the Hierta Retzius and Lars Hierta's [illegible] I am [illegible] indebted to

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Acta physiol. scand. 1966. 68. 18 — 22

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Effect of Phenoxybenzamine and Hydergine on Urinary Catechol Amines in Rats during Restraint

By

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Received 3 August 1965

Abstract

GRAHAM, LINDSAY A *Effect of phenoxybenzamine and Hydergine on urinary catechol amines in rats during restraint* Acta physiol scand 1966 *68* 18—22

output of A in urine during restraint

Physical restraint has been used for many years by Selye (1958) to produce stress in animals It would be expected that the stress of muscular restraint would be associated with both increased neurogenic sympathetic and adrenomedullary activity.

This paper reports an initial investigation in which urinary adrenaline (A) and noradrenaline (NA) were measured in restrained rats Furthermore, the sympathetic alpha-blocking agents phenoxybenzamine (PBA) and Hydergine were administered and the effect on urinary catechol amine excretion observed

Materials and Methods

Male Sprague Dawley rats of about 150 g were used Control (free) rats were kept in individual metabolism cages without food or water concurrently with restrained rats Experimental rats were

Samples from free rats were paired those from restrained rats analyzed individually Catechol

In an initial

found to be

more suitable

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TABLE I Effect of PBA and atropine on the urinary catechol amine excretion rates (µg/kg/hr) of free and restrained rats Numbers of samples in each group are in parentheses

		Saline		PBA 10 mg/kg		Atropine 15 mg/kg	
Free	NA	0 11	(4)	0 75	(6)		
	A	0 03		0 17			
Restrained	NA	0 31	(10)	1 01	(8)	0 24	(7)
	A	0 28		0 51		0 28	

Tests of differences between means for restrained rats

PBA vs Saline	NA	$p<0.01$
	A	$p<0.05$
Atropine vs Saline	NA	NS
	A	NS

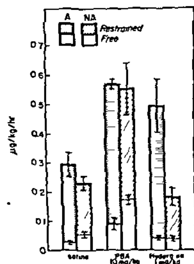


Fig 1 Effect of PBA and Hydergine on free and restrained (16 hrs) rats S E of mean is indicated for each group Minimum n = 6 maximum n = 10

Hydergine (Sandoz S A Basle Switzerland) and phenoxybenzamine as Dibenzyline (Smith Kline & French) in injectable solution were given in single intramuscular injections in the leg immediately prior to restraint Control animals were injected with equivalent volumes of saline and some restrained animals were given atropine sulphate The order of drug injections was randomized

Results

Effect of PBA and atropine on rats restrained for 12 hrs

Rats restrained for 12 hrs were injected with PBA 10 mg kg Atropine sulphate (15 mg/kg) was given to restrained rats to compare the effect of PBA with a vagal blocking agent The effects of these drugs on urinary catechol amine excretion rates are shown in Table I

TABLE II Effect of NA loading (100 μg/kg) on the urinary excretion of NA (μg/kg/hr) in PBA and Hydergine treated rats Number of rats per group = 6 Urine collection time 16 hrs

	Saline		PBA 10 mg/kg		Hydergine 1 mg/kg	
	A	NA	A	NA	A	NA
Control	0 03	0 06	0 09	0 17	0 05	0 04
NA loading	0 03	0 15	0 12	0 60	0 04	0 20
Recovery (%)		1 4%		6 9%		2 6%

There was an increased excretion of both A and NA during restraint, and a marked increase in NA in both free and restrained PBA-treated rats Adrenaline excretion was also greater in these animals There was no difference in excretion of either amine between control and atropine-treated groups However, because of some difficulty with 12 hrs urine collections, the effect of PBA was repeated in rats restrained for 16 hrs

Effect of PBA and Hydergine on rats restrained for 16 hrs

Free and restrained rats were given PBA 10 mg/kg or Hydergine 1 mg/kg The effect of these drugs on the catechol amine excretion rates is shown in Fig 1

Restraint caused a significant increase in excretion of both amines Furthermore, PBA caused an increased excretion of both amines, particularly NA, in free rats, while Hydergine-treated free rats were no different from controls In restrained rats PBA caused a marked and significant increase in the excretion of both amines compared to restrained controls The effect of Hydergine was to cause an increased A excretion with no change in NA excretion rate

Effect of NA loading on PBA and Hydergine treated rats

The difference in NA excretion between PBA and Hydergine-treated rats could be due to greater release of NA at the nerve endings by PBA, or to inhibited destruction of released NA, perhaps due to blockage of "metabolic" as well as "effector" receptors To test the latter hypothesis, a loading dose of NA (100 μg/kg) was given to rats pre-treated by 1 hr with both drugs Urine was collected for 16 hrs, with the animals in the free state Recovery values were calculated on the basis of control values obtained from the same rats treated identically on the previous day, but without NA injections (Table II)

The recovery of 2 6 % of the injected NA in Hydergine-treated animals compares with that of 1 4 % for control animals However, the 6 9 % recovery found for PBA-treated rats suggests an interference in the inactivation of circulating NA by this drug

Discussion

The increased A and NA excretion rates in restrained rats indicated that the stress of physical restraint caused marked increases in both adrenomedullary and neurogenic sympathetic activity. Thus the technique of restraining rats appeared to be a simple and effective way to study catechol amine metabolism during stress.

Phenoxybenzamine caused an increased excretion of both amines in free rats, compared to controls. Schapiro (1958) has also found increased NA excretion with this drug, in similar dosages but found no change in A levels. It is possible that the difference is due to the chronic nature of Schapiro's study, compared to the acute values reported here.

With restraint the increase in the excretion of both amines observed in rats treated with PBA was still further accentuated. In the experiment with Hydergine, only the A excretion was increased. It seems likely that the effect of restraint compounded with the effect of these drugs caused a large adrenomedullary release of adrenaline. One reason for this may have been the general physical state of the animals since some, especially those treated with PBA, appeared to have lower body temperature and metabolic rates.

The increased NA excretion in restrained, PBA treated animals was possibly due to an interference with the re-uptake of NA or inactivation of NA, either at metabolic sites near the nerve endings or in the liver, as suggested by Schapiro (1958). That such was, in part, the case was suggested by the greater excretion of an injected dose of NA in PBA treated animals. Such an effect did not occur with Hydergine. The possibility that PBA also caused a greater release of NA from the nerve endings cannot be excluded.

Both PBA and Hydergine have been shown by Brown and Gillespie (1957) to increase the output of NA from the spleen of the cat, when the splenic nerves are stimulated. These authors suggest that the blocking agent prevents the uptake by the splenic tissue of the liberated transmitter. Experiments by Blakeley, Brown and Ferry (1963) suggest that a proposed alternative hypothesis is unlikely, namely that these adrenergic blocking agents act on a cholinergic link in the post ganglionic pathway (Boyd, Chang and Rand 1960). Furthermore at low frequencies of stimulation (less than 10/sec) the removal of liberated transmitter appeared to be complete (Brown and Gillespie 1957). Also Gillespie and Kirpekar (1963) have shown that when the cat spleen is perfused with NA, PBA has a consistently greater capacity to prevent uptake of the infused NA into the spleen than does Hydergine.

It is likely, therefore that in rats pre-treated with PBA recovery of infused NA was greater because of block by the drug of uptake at the nerve endings. The experiments of Gillespie and Kirpekar suggested that Hydergine would be less effective in this respect than PBA. That Hydergine appeared to have no effect at all may have been due to the dosage used. On the other hand, since the animals were at rest the rate of spontaneous nerve stimulation may have been sufficiently low to allow similar uptake of both the endogenous and exogenous NA in the Hydergine tr

and control groups Alternatively, different metabolic processes of NA might occur under the influence of the two drugs, with PBA being more effective in inhibiting the breakdown of NA in the liver, as suggested by Schapiro (1958)

This study was supported in part by funds provided by Special Fellowship 5 F3 MH 22 481 02 of the National Institute of Mental Health The technical assistance of Mrs Anita Jorgensen was greatly appreciated

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Acta physiol. scand 1966 68. 23 — 27

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Surface Properties of Lung Extracts

I. A Dynamic Alveolar Model

By

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Received 29 August 1965

Abstract

ADAMS, F H and G ENHORNING *Surface properties of lung extracts* I *A dynamic alveolar model* Acta physiol scand 1966 *68* 23—27

The importance of surface properties in the layer lining the alveoli is now well established For measuring these properties a dynamic model of the alveolus has been constructed It has the following advantages over previous techniques used to study the surface tension of biological fluids including lung extracts

1 It more closely simulates the physiologic changes in rate and area that occur in the lungs during respiration.

2 Only a small volume of fluid (0 2 ml) is required

3 The surface tension can be determined in 30 min or less

It is suggested that the visco-elastic properties of the alveolar surface film may be important as well as the surface tension in determining the response to compression and expansion

Over the past 10 years, there has been an increasing interest in the role of surface forces in the function of the lung in health and disease Surface tension is a potential hindrance to lung expansion after birth and it tends to cause expanded alveoli to collapse again

Von Neergaard in 1929 suggested that the surface tension of alveoli might be lower than that of other body fluids due to accumulation of surface active material He used the Lenard method for measuring surface tension which is similar to that of du Nouy (1926) These methods as well as the stalagmometric (Agostoni *et al* 1958) are suitable for evaluating how surface tension resists lung expansion after birth when hemispherical air liquid interfaces move down the airways Recently this problem has also been studied with a modification of the maximal bubble pressure method which simulates the movement of an air liquid interface through an airway (Enhorning 1964, Enhorning and Kirschbaum 1964

Due to surface tension expanded alveoli tend to collapse but are protected by a lining layer The significance of the ability of the lung lining layer to form stable

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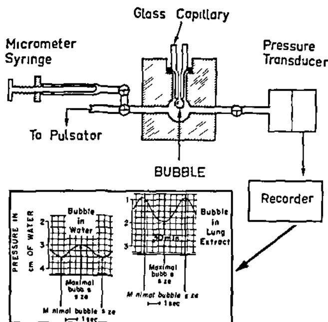


Fig 1 Principle for studying surface properties with a dynamic alveolar model A bubble communicates with atmosphere Since bubble radius is measured through a microscope and since the pressure around the bubble is recorded surface tension can be calculated Bubble size is adjusted with micrometer syringe

am was neglected until Pattle (1955) became interested in antifoam agents He found that small bubbles expressed from cut lungs and observed through a microscope remained stable over 20 min He thus concluded that the surface tension lining the bubble was very low, less than 0 06 dyn/cm The method described by Pattle has been used successfully in an important clinical study (Pattle *et al* 1962) However, it does not allow re expansion of the film outlining the bubble and it is 'difficult to get reproducible results for the rate of contraction of bubbles" (Pattle 1965)

Many investigators studying the surface tension of the lung lining layer use the modified Wilhelmy balance (Clements, Brown and Johnson 1958) This instrument, however, requires a large volume of fluid, 50—70 ml, and the flat surface film developed in its trough cannot be compressed and expanded at the same rate as the alveolar film during respiration

Since the importance of surface properties in the layer lining the alveoli is now well established, there is a need for a method which permits a fast evaluation of the surface properties and requires only a small sample volume Furthermore the method should simulate the surface changes taking place in the alveolus during breathing These points were taken into account when constructing a dynamic alveolar model for studying surface properties Some experiences with this instrument have already been reported (Enhorning Fujiwara and Adams 1964, Enhorning and Adams 1965) but a detailed description of the method is given in this paper

Description of the apparatus

Fig 1 presents the principal for studying surface properties with a model of an alveolus a bubble which communicates with atmosphere and which can be made to change in size cyclically A test chamber, with a volume of 0 2 ml containing the sample communicates with the atmosphere via

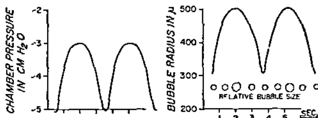


Fig 2 (Above) Tracing obtained when bubble was pulsating in water (Below) Since there was a constant surface tension of 72 dyn/cm, bubble radius could be calculated with La place formula

a vertical glass capillary The chamber is also connected with a micrometer syringe, a pulsator unit, and a pressure transducer (Sanborn Model 268 A) The system is first completely filled with liquid

moved in and out of the system at a frequency that can be varied from 10 to 180 cycles/min The

$= 2\gamma/r$ the surface tension of the sample (γ) can be calculated since ΔP and the radius (r) are known In the formula ΔP is in dyn/cm², and consequently the value read from the record in cm of water is multiplied by 980 Since the radius is measured in μ but is in cm in the formula it is multiplied by 10^{-4}

Surface properties as reflected by pressure recording

Fig 1 and 2 show pressure tracings obtained when a bubble is made to pulsate in water The stroke

to pulsate in water or in saline solution surface tension at the air liquid interface remains at a constant value of approximately 72 dyn/cm which is the surface tension of water at 20° C Since surface tension of water is constant the pressure tracing makes it possible to calculate how the bubble radius changes continuously during the pulsator cycle (Fig 2)

When the water in the test chamber is replaced with an extract from normal adult lung (Clements 1962) there is a reduced tension in the surface of the air bubble This conclusion is drawn from the fact that the pressure gradient across the bubble surface is less even when the bubble is first expanded During the following minutes while the bubble is maintained at a fixed radius the surface tension becomes further reduced This latter observation implies that in accordance with Gibb s law surface active material is moving to the surface and thereby reduces the surface tension With a bubble radius of 500 μ the pressure gradient ΔP finally reaches a value of slightly less than 2 cm of water From these figures surface tension can be calculated to be approximately 40 dyn cm When the bubble is made to disappear and is replaced with a new one of equal size the pressure gradient across the surface of this new bubble is again reduced according to the same pattern and reaches the final value of slightly less than 2 cm of water When the pulsator unit is started causing the bubble volume to oscillate the pressure tracing is immediately conspicuously different from that obtained with water or saline solution and changes further with time (Fig 1) Whereas in water the product of the pressure gradient (ΔP) and the radius (r) remains constant indicating a con

chronously the product of these two entities which is proportional to surface tension γ oscillates greatly By inserting corresponding values of ΔP and r into the formula of Laplace the maximal value of γ at maximal bubble size can be calculated as can the minimal value of γ which appears at minimal bubble size

Discussion

The alveolar model described above confirms the observation made with the modified Wilhelmy balance (Clements *et al* 1958) that area compression of a lung extract film results in a reduction of surface tension A lesser reduction of surface tension can be correlated with conditions characterized by a tendency to develop atelectasis (Avery and Mead 1959, Gruenwald *et al* 1962) an observation which may be given the following explanation

At the end of expiration alveolar surface area is maximally compressed, since alveolar radius (r) is minimal at that time From the formula of Laplace, $\Delta P = 2\gamma/r$ it is clear that if there is a substantially decreased surface tension (γ) when r is minimal the pressure gradient across the alveolar surface (ΔP), which is necessary for alveolar expansion, may also be minimal However, with a lesser reduction of γ there may be no reduction but an increase of ΔP To maintain alveolar expansion in case of an increased ΔP, intrathoracic pressure has to be more negative At the end of expiration it is least negative and may not be sufficiently reduced to yield the necessary value of ΔP As a result many alveoli particularly the smallest, would tend to shrink and collapse whereas the remaining large alveoli would become even larger This explains the typical histological picture of lungs from newborns dying of idiopathic respiratory distress areas of atelectasis alternating with areas of emphysema (Gruenwald 1956 1958) However, if γ decreases extensively during expiration, the ΔP required to maintain alveolar expansion would also decrease and would correspond to the existing negative intrathoracic pressure Recording of pressure with the alveolar model demonstrates that as the alveolus becomes smaller the pressure gradient required to keep it expanded becomes less This illustrates the stabilizing ability of the surface film

It is of interest to note that the calculated surface tension at the interface of the bubble pulsating in lung extract oscillates around the value of surface tension at the interface of a non pulsating bubble It would seem that the surface active material which becomes concentrated in the air liquid interface not only reduces surface tension, but it also forms a film with visco-elastic properties This film offers resistance to any change in bubble size and when the pulsator unit moves liquid into the test chamber thereby forcing the bubble to become smaller, the surface film offers resistance to such a change As a result there is an increase in pressure of the liquid surrounding the bubble When the pulsator unit withdraws liquid from the test chamber, this is also resisted by the surface film Consequently the negative pressure around the bubble becomes more negative than would be required to counteract the bubble shrinking action of surface tension alone This interpretation implies that not only a reduced surface tension is required to maintain the alveolar stability but also other properties of the alveolar film may be of importance To prevent collapse

of the recently expanded alveoli of the newborn infant, it might be of importance not only that surface tension is reduced quickly but also that other physical properties, such as viscosity and elasticity, develop rapidly

Supported in part by grants from the United Cerebral Palsy Research and Educational Foundation (Dwight D Eisenhower Fund) and the United States Public Health Service

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Acta physiol scand. 1966 68 28 — 36

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Surface Properties of Lung Extracts

II. Comparison of Fetal and Adult Rabbits

By

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Received 29 August 1965

Abstract

ADAMS, F H, G ENHORNING and A NORMAN Surface properties of lung extracts II Comparison of fetal and adult rabbits Acta physiol scand 1966 *68* 28 —36

compressed the tension decreased and when the surface area was expanded the tension increased The difference between the mean value and the minimal or the maximal value was a function of change in surface area as well as time The change in surface area and the rate with which it occurs in the lungs is more closely simulated with the dynamic alveolar model than with the modified Wilhelmy balance The minimal value of surface tension was recorded at minimal surface area It increased if surface area was maintained at minimal size This demonstrates viscosity of the surface film For alveolar stability the outlining film should probably have high viscosity as well as low surface tension In the newborn these properties have to develop rapidly to prevent alveolar collapse

The properties of the lung lining layer obtained by various methods appear to be important in health and disease (Pattle 1965, Avery and Said 1965) Pulmonary surfactant has been reported to appear during fetal life after 18 days in the mouse (Pattle 1961, Buckingham and Avery 1962) and 120 days in the sheep (Adams and Fujiwara 1963) In human fetuses it has only been found in those weighing more than 1 000 g (Avery and Mead 1959) Gruenwald on the other hand, has shown that the lungs of some very young human fetuses possess high surface activity and display normal pressure volume curves (Gruenwald 1963)

The question remains however, are the surface properties of lung extracts of term fetal animals identical with those obtained from normal adult animals? The results of the investigations to be reported indicate that the surface tension of lung

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TABLE I Surface tension (dyn/cm, mean ± S D) in nonpulsating bubble as a function of time

	n	1/4 min	1 min	2 min	30 min
Fetal lung extract (3 g tissue/50 ml)	6	57±4	55±3	53±2	49±3
Adult lung extract (3 g tissue/50 ml)	8	48±6	47±6	45±7	41±5
Difference±S E		9±2 7	8±2 8	8±2 6	8±2 2

S D = Standard deviation S E = Standard error

extracts obtained from fetal rabbits at term is higher than that obtained from adult rabbits and that other properties of the lung lining layer may also be of importance

Material and Procedure

Eleven rabbits were used for this study Six were pregnant and were within three days from term All animals were sacrificed by intracisternal injection of 3—5 ml of 2 % Xylocaine The lungs were carefully removed for preparation of extracts which were then studied with the dynamic alveolar model previously described (Adams and Enhorning 1965) and with the modified Wilhelmy balance (Brown Johnson and Clements 1959, Clements 1962) Of the extract the dynamic alveolar model required 0 2 ml and the trough of the modified Wilhelmy balance 50 ml To obtain sufficient material the lungs from all fetuses of one litter were pooled This gave 5 samples of fetal lung extract each large enough to be studied by both methods However one rabbit had only two fetuses and the extract in this case was sufficient only for a study with the dynamic alveolar model

The extracts from all ...

per 1 ml of ...

tracing indicated a clean chamber

After calibration at — 1 and — 3 cm of water the saline solution was replaced with the unknown sample which was withdrawn with the micrometer syringe until a bubble with a radius of 500 μ was formed While the bubble was maintained at this size the chamber pressure slowly became less negative In a ...

whereby a new bubble was formed Its radius was then changed cyclically from a maximum of 500 μ to a minimum of 425 μ as the pulsator unit moved 0 2 mm^3 of liquid in and out of the bubble chamber For at least 30 min the pressure was recorded continuously while the pulsator was maintained at the same frequency and volume displacement Cycling rates up to 180 min were also tested after which the pressure was recorded while the bubble radius was maintained stationary at 500 ...

study the minimal value of surface tension for a given sample was defined as the lowest value obtained during a 16 hour recording and the maximal value as the highest value noted during this time

Ten extracts of adult lungs (8 of normal concentration 2 of higher concentration and 5 extracts of fetal lungs were studied by both methods The extract of the lungs from the eleventh rabbit was examined in a different manner using the Wilhelmy balance) The surface area was not only compressed to 20 % of its maximal size but also to approximately 40 %, 60 % and 80 %

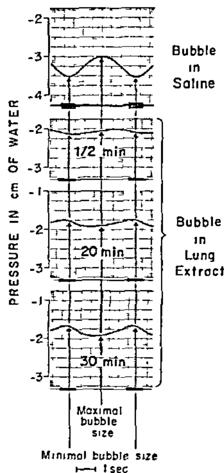


Fig 1

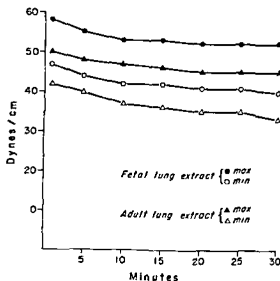


Fig 2

Fig 1 Pressure tracing obtained when bubble was pulsating in fetal lung extract
Fig 2 Mean surface tensions at maximal and at minimal bubble size showing progressive decrease with time

Results

Table I contains the results of the surface tension measurement as a function of time in a nonpulsating bubble from fetal and adult lung extracts The mean fetal values obtained at 1 2, 1 and 2 min after formation of the bubble and after 30 min pulsation were significantly higher ($P < 0\ 01$) than the corresponding extracts from adult lungs

When the pulsator unit was turned on, a very characteristic pressure tracing was obtained with saline solution in the sample chamber The pressure was most negative (ΔP_{max}) at the time when the bubble was smallest

As soon as the saline solution had been replaced with lung extract the pattern of the pressure tracing was different Fig 1) The most negative pressure no longer coincided with minimal bubble size Instead, it occurred while the bubble was

TABLE II Surface tension (dyn/cm mean±S D) in pulsating bubble as a function of time

	n	1 min		5 min		10 min	
		max	min	max	min	max	min
Fetal lung extract (3 g tissue/50 ml)	6	58±2 3	47±1 7	55±3 8	44±1 4	53±2 4	42±2 0
Adult lung extract (3 g tissue/50 ml)	8	50±4 2	42±2 9	48±4 7	40±4 4	47±4 3	37±4 2
Difference±S E		8±1 8	5±1 2	7±2 7	4±1 7	6±2 0	5±1 7

S D = Standard deviation S E Standard error

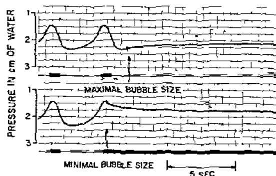


Fig 3 Tracings obtained when pulsator was stopped at maximal and minimal bubble size showing spontaneous change in pressure toward the mean

becoming larger The tracing of a newly formed bubble, pulsating in lung extract was slightly skewed, but with continued recording it became more symmetrical, and the pattern finally became almost the inverse of that of saline i e the most negative pressure (ΔP_{max}) preceded and almost coincided with maximal bubble size Thus, the apparent value of surface tension was minimal when the bubble was smallest and maximal when the bubble was largest These extreme surface tension values for a pulsating bubble were above and below that obtained with a nonpulsating bubble The maximal and particularly, the minimal apparent surface tension decreased as a function of time as seen in Table II and Fig 1 and 2 The fetal values remained higher than those of the adult, and the difference between them after 30 min of pulsating was highly significant ($P < 0.01$)

When the speed of the pulsator unit was increased there was no change in maximal or minimal pressure with saline solution in the sample chamber but with a lung extract there was an increase in pressure amplitude With an extract of fetal origin and with a frequency change from 17 to 180 cycles/min the amplitude increased by 69 ± 23 % (mean±S D With an extract of adult lungs the increase was 46±5 % (mean±S D) with the same frequency change The difference between the two lung extracts was 23 % with a standard error of 10 % The amplitude increase affected maximal and minimal pressure values equally When the pulsator

15 min		20 min		25 min		30 min	
max	*min*	*max*	*min*	*max*	*min*	*max*	*min*
53±2.5	42±1.8	52±2.4	41±2.3	52±2.7	41±2.4	52±2.5	40±3.2
46±4.3	36±4.0	45±3.8	35±3.5	45±3.2	35±3.8	45±3.3	33±4.2
7±1.5	6±1.5	7±1.7	6±1.5	7±1.6	6±1.7	7±1.6	7±1.9

TABLE III Surface tension (dyn/cm, mean±S.D.) as it appears when surface area is compressed and expanded in trough of Cap Wilhelmy balance and in pulsating bubble

	Fetal lung extract (3 g tissue/50 ml)		Adult lung extract (3 g tissue/50 ml)	
	max	min	max	min
Wilhelmy balance	69±3.4 n 5	24±3.5 n=5	47±7.4 n=8	12±1.5 n 8
Bubble chamber	52±2.5 n=6	40±3.2 n 6	45±3.3 n=8	33±4.2 n=8

S.D. Standard deviation.

unit was turned off, either at maximal or at minimal bubble size, the apparent surface tension tended to approach the mean value as shown in Fig. 3

The results thus far given were obtained with the extracts prepared with 3 g lung tissue per 50 ml of saline solution. With the more concentrated extracts (6 g tissue/50 ml) prepared from the lungs of 2 adult rabbits the tracings showed the same pattern but the values for apparent surface tension at minimal bubble size decreased faster and were lower. After 30 min of pulsation, these values for the two rabbits were 29 dyn/cm, and 28 dyn/cm respectively.

With the Wilhelmy balance the minimal value of surface tension in fetal lung extract was 24 ± 3.5 dyn/cm (mean ± S.D.) whereas in adult lung extract the value came down to 12 ± 1.5 dyn/cm (mean ± S.D.). The difference 12 dyn/cm has a standard error of less than 2 dyn/cm and is highly significant ($P < 0.01$).

As seen in Table III the minimal values recorded with the modified Wilhelmy balance were lower and the maximal values higher than those obtained with the dynamic alveolar model. However, when there was less reduction of the surface area in the trough of the Wilhelmy balance, the minimal value increased and the maxi

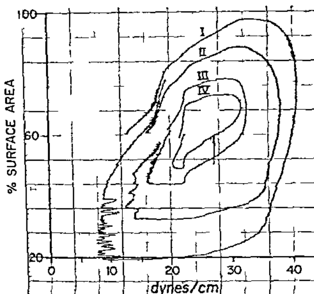


Fig 4

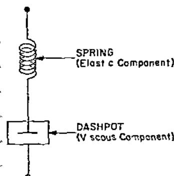


Fig 5

Fig 4 Tracing obtained with modified Wilhelmy balance when surface area was reduced to 20 % (I) 40 % (II) 60 % (III) and 80 % (IV) of original size Results show effects of change in film compression but may also show effects of ageing since IV was made first and I last

Fig 5 Maxwell element

mal value decreased so that the result became more similar to that obtained with the dynamic alveolar model (Fig 4)

Discussion

Difference between adult and fetal lungs With both methods used in this study it was found that the extracts showed more surface activity when they originated from adult than from fetal rabbit lungs This probably reflects a difference in concentration of surface active material since more concentrated extracts prepared from 6 g instead of 3 g tissue per 50 ml yielded lower values of surface tension at minimal surface area However in view of the demonstration by Levine and Johnson (1964) that surface active material is more readily extracted from aerated than from atelectatic lungs it would seem unsafe to conclude that also in lungs of adult rabbits is there a higher concentration of surface active material Nevertheless there is reason to believe that aeration of the lungs after birth will promote a concentration of surfactants in all the air liquid interfaces produced in the alveoli Such a movement of surfactants to the surface would be in accordance with Gibbs law and is demonstrated to occur in the dynamic alveolar model by the increase in pressure amplitude during the 30 min of recording Fig 1 Thus as the surfactants are being produced by alveolar cells or as they arrive to the lungs by way of the blood stream they will have a tendency to remain in the air liquid interface and their concentration if insufficient at birth may finally become adequate This might explain why in

many cases of respiratory distress in the newborn there is a spontaneous regress of the symptoms

Influence of surface compression on minimal value of surface tension The very low value of surface tension characteristic for a normal lung extract is recorded with the modified Wilhelmy balance when the surface film is compressed to approximately 20 % of its maximal size With less compression the value is higher (Fig 4) If this is analogous to *in vivo* conditions the very low value of surface tension is reached only if the air liquid interface of the alveolus is compressed to 20 % of its maximal size Yet according to Pattle it is conceivable that respiration causes no surface area reduction He visualizes the alveolus as a wrinkled paper bag which may be blown up without any change of its inner surface area (Pattle 1963) Usually however, the alveolus is thought of as a spherical bubble blown at the end of a narrow tubing

Assuming the alveolus has a spherical shape it can be calculated how breathing changes its surface In a young healthy man the tidal volume is about 600 ml and the functional residual capacity 2 400 ml (Comroe *et al* 1962) The volume at the end of normal expiration is therefore 4/5 of that at the end of normal inspiration Since the volume of a sphere is proportional to the third power of its radius the alveolar radius at the end of expiration will be what it is at the end of inspiration multiplied by $\sqrt[3]{4/5}$ The surface of a sphere is proportional to the square of its radius and therefore the alveolar surface at the end of expiration will be what it is at the end of inspiration multiplied by $[\sqrt[3]{4/5}]^2$ Thus at the end of expiration the surface area has been reduced to 86 % of what it was at the end of a normal inspiration With deeper breathing the reduction in surface area may be greater but even at maximal expiration in young healthy men the surface area is compressed only to 34 % of its maximal size This figure is derived by assuming that the residual volume is 20 % of the total lung capacity However in men 50 to 60 years old residual volume is 40 % of total lung capacity (Comroe *et al* 1962) and under those circumstances the maximal surface area reduction will be 54 % These calculations are in agreement with those of Storey and Staub (1962) They measured diameters of alveoli in rapid frozen cat lungs and calculated that the surface area could be increased 70 % which means that the minimal area is 60 % of the maximal In view of these estimations of how the alveolar surface area changes during breathing the surface of the alveolus in the dynamic model at end of expiration was chosen to be approximately 70 % of its maximal size

With normal breathing the changes in alveolar surface area are hence quite small and the true values of surface tension are probably not as high as the maximal and not as low as the minimal values recorded with the Wilhelmy balance The true values oscillate around a mean obtained when there is no change in surface area The film outlining the alveolus lowers surface tension but it also seems to resist any change in size To keep the alveolus expanded in the lung extract a negative pressure had to be exerted But when the alveolus was made smaller (expiration) the required negative pressure decreased as if the surface film opposed

the change and to a certain extent neutralized the effect of surface tension When on the other hand the "alveolus" was made larger (inspiration) the surface film seemed to resist this change too Therefore a greater negative pressure had to be exerted than would be needed to overcome the effect of surface tension alone

Influence of cycling frequency on minimal value of surface tension The minimal value of surface tension, recorded during maximal reduction of surface area, is a function not only of this area but also of time This observation confirms what has previously been reported (Sutnick and Soloff 1963) If the area is maintained at minimal size the recorded value of γ will increase, and if maintained at maximal size it will decrease When a lung extract was examined with the alveolar model the pressure amplitude became greater with increasing rate of pulsation As originally pointed out by Avery and Mead (1959) it is as if visco-elastic properties had been formed in a surface film

A biological visco elasticity may be illustrated with a Maxwell element which consists of a spring, representing the elastic component, in series with a dashpot, representing the viscous component (Stacy *et al* 1955) (Fig 5) A dashpot consists of a container with oil in which a disc moves in a direction which is perpendicular to its surface If there is a sudden increase in length of the Maxwell element, this will be taken up entirely by the spring, since the dashpot requires time to move According to Hooke's law the increase in tension is proportional to the increase in length of any element which is elastic However, with time, the dashpot will start moving, and this will diminish the stretching of the spring and thus its tension The dashpot will move more slowly, the decrease in tension being exponential The higher the viscosity of the viscous component, the longer it takes for the spring action to be reduced

When alveolar' pulsations were suddenly stopped at maximal or minimal 'alveolar" size or when the barrier of the modified Wilhelmy balance was stopped at maximal or at minimal surface area, there was an apparent exponential decrease in stretching of the elastic surface film component (Fig 3 of this article and Fig 6 of paper by Sutnick and Soloff 1963) This decrease in stretching would go faster with low viscosity in the surface film The increase in pressure amplitude with in creased cycling frequency of the alveolar model suggested that at the low frequency of 17 cpm there was time to allow a reduced stretching and compression of the elastic surface film component as the bubble approached maximal and minimal size, respectively Thus, the increase in pressure amplitude observed particularly with the extracts of fetal origin would seem to reflect low viscosity of the film out lining the bubble At low frequency the viscous component, "the dashpot had time to move and the stretching of ' the spring was reduced

Breath holding at maximal expiration could jeopardize alveolar expansion if viscosity of the alveolar film is low This would allow rapid disappearance of the spring action, i e a fast increase of surface tension Thus it would seem conceivable that for alveolar stability the film outlining the alveolus should have not only low surface tension but also high viscosity In the mature fetus these properties

may not be fully developed but they have to develop rapidly in the newborn in order to protect the just expanded lungs from collapsing again

Supported in part by grants from the United Cerebral Palsy Research and Educational Foundation (Dwight D Eisenhower Fund) and the United States Public Health Service

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Acta Physiol. scand. 1966. 68. 37 — 42

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Surface Properties of Lung Extracts

III. Changes Appearing during First Ten Minutes after Surface Formation

By

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Received 29 August 1965

Abstract

Adams, F H, G Enhörning and M Regne Karlsson *Surface properties of lung extracts. III Changes appearing during first ten minutes after surface formation* Acta physiol scand 1966 *68* 37—42

Surface properties of washings and extracts from fetal guinea pig lungs were investigated with the dynamic alveol [illegible]
var [illegible]
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time After 2 min the mean values were significantly higher in lung extracts from fetuses weighing more than 60 g than in lung extracts from fetuses weighing less — Thus after only 2 min of measurement and with a small sample (0 2 ml) the method makes it possible to differentiate immature from more mature lung surface properties The method might also make it possible to differentiate normal infants from those who subsequently develop idiopathic respiratory distress by examining the fluid obtained by aspiration of the upper airways

Idiopathic respiratory distress of the newborn is still a major cause of neonatal mortality (Ahvenainen 1959, Arey 1960, Avery and Oppenheimer 1960) A prominent feature of the condition is collapse of many alveoli Gruenwald 1956, Briggs and Hogg 1958) For alveolar stability, surface tension has to decrease extensively during expiration and increase during inspiration (Brown Johnson and Clements 1959), and according to Avery and Mead (1959 and Pattle *et al* 1962) this important surface property is deficient in most infants dying from idiopathic respiratory distress To prevent alveolar collapse the surface properties must develop rapidly probably within the first few minutes after initial aeration

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TABLE I Surface tension (dyn/cm) in a nonpulsating bubble in lung extract as a function of time (mean ± S D)

Group no	Weight	n of subjects	1 min	2 min	10 min
1	<60 g	17	49 2±4 0	47 4±4 0	33 4±4 0
2	>60 g	21	46 8±4 2	41 8±4 2	33 1±4 0
1+2	3 4—135 g	38	47 9±2 9	44 2±3 1	33 3±2 9
Difference between groups 1 and 2 (D±S E)			2 4±2 6	5 6±2 6	0 3±2 6

S D = *Standard deviation* *S E* = *Standard* error

This paper concerns the surface properties of washings and extracts from fetal guinea pig lungs determined with the dynamic alveolar model previously described (Adams and Enhorning 1966) Special attention has been given to the early changes in the air-liquid interface and to the correlation of these changes with fetal weight

Materials and Methods

fetuses were lost due to technical difficulties

The pregnant guinea pigs were sacrificed by intracisternal injection of 2—5 ml of 2 % Xylocaine Immediate laparotomy was performed and before delivering the fetuses their necks were ligated to prevent aspiration of amniotic fluid Fetal weight was recorded in each case

For this study the pulsator of the dynamic alveolar model was adjusted to alter the "alveolar

formed The bubble was maintained at this size for 2—3 min It was then extruded and rep with a new bubble which pulsated at 17 cpm for at least 10 min Finally the pulsator was stopped and the bubble was made to remain stationary at a radius of 500 μ for an additional 2—3 min

1966)

As a way of expressing the stabilizing ability of the surface film the stability index $\overline{S}$ was calculated using the formula

$$\overline{S} = \frac{2(\gamma max - \gamma min)}{\gamma max + \gamma min}$$ (Clements *et al* 1961

sure coincided with the mark in sample the most negative pressure e which reflects viscosity was ex was 360 degrees

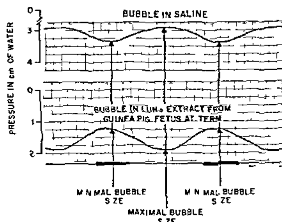


Fig 1 Pressure tracings obtained when bubble pulsated in saline and in lung extract In both liquids bubble radius changed from a maximum of 500 μ to a minimum of 450 μ

Results

With a nonpulsating bubble in lung washings, surface tension (mean ± S D) after 1, 2 and more than 10 min was 61 4 ± 2 6, 59 9 ± 2 2, and 56 2 ± 3 5 dyn/cm respectively The values for lung washings were high for both mature and immature fetuses but for lung extracts they were lower (Table I) The surface tension decreased faster when the extract was derived from fetuses weighing more than 60 g

With a pulsating bubble in lung washing or lung extract, the most negative chamber pressure, thus the maximal value of ΔP, no longer coincided with minimal bubble size as it did when there was saline in the test chamber (Fig 1) Instead ΔP_{max} occurred while the bubble was becoming larger The phase difference was apparent already at beginning of bubble pulsation but increased with duration of cycling and finally approached 180° Thus after 10 min surface tension decreased as the bubble became smaller, and increased as the bubble became larger Since pressure amplitude as well as phase difference increased with time, the difference between the maximal and the minimal value of surface tension also increased as a function of time After 10 min the maximal and minimal value of surface tension (mean ± S D) was 61 0 ± 2 6 and 46 4 ± 2 9 dyn/cm in washings and 46 4 ± ± 3 5 and 27 9 ± 3 7 dyn/cm in extracts

The decrease of the maximal and particularly the minimal value of surface tension during the 10 min of recording is reflected in an increase of stability index After 2 min the mean value for lung washings was 0 24 ± 0 02 and after 10 min 0 27 ± 0 05 The 2 min value of lung washings did not change with fetal weight as did the value of lung extracts Two minutes after bubble formation the stability index was still below 0 30 in each of the extracts from fetuses weighing less than 60 g but was above this value in extracts from 13 of the 21 fetuses weighing more than 60 g (Fig 2) Table II shows the increase in the 2 min value of stability index with increase in fetal weight The differences between the values of group 2 and 3 of this table (0 22) has a standard error of 0 07 and is significant $P < 0\ 01$

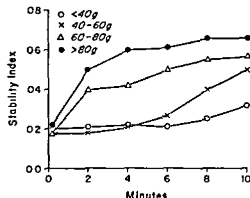


Fig 2 Change of stability index with time Mean values for fetuses in different weight groups Already after 2 min the index was significantly higher for fetuses weighing more than 60 g than for those weighing less

TABLE II Stability index and phase difference two minutes after bubble formation in lung extracts

Group no	Fetal weight	n of subjects	$\bar{S}$ (mean ± S D)	Phase difference in degrees (mean ± S D)
1	<40 g	8	0 21 ± 0 04	125 ± 4 6
2	40—60 g	9	0 18 ± 0 07	120 ± 6 2
3	60—80 g	9	0 40 ± 0 15	150 ± 16 5
4	>80 g	12	0 45 ± 0 15	150 ± 13 2
Difference between groups 2 and 3 (D ± S E)			0 22 ± 0 07	30 ± 8

S D —Standard deviation S E — Standard error $\bar{S}$ Stability index

Phase difference increased slowly in lung washings but rapidly in lung extracts Two minutes after bubble formation, it had the same value in lung washings from fetuses weighing less than 60 g as from those weighing more In lung extracts from 12 of 21 fetuses weighing more than 60 g, the 2 min value of phase difference had become greater than 140°, but in none of the extracts from fetuses weighing less than 60 g had the phase difference reached this value Table II demonstrates how the phase difference after 2 min was greater in lung extracts from the heavier fetuses The difference between groups 2 and 3 (30°) has a standard error of 8° and is significant ($P < 0.01$)

Discussion

The results obtained in this study indicate that lung extracts contain more surface active material than do airway washings In view of previous studies (Levine and Johnson *1964, Enhörning Fujiwara and Adams 1964, Adams et al 1965) it is likely* that the higher minimal value of surface tension, observed in the washings, is due to a low concentration of active phospholipids rather than to a change in their composition With the technique used for washing the airways, there might have been an

incomplete removal of surface active material from the alveoli In the fetal lamb there is a continuous flow of fluid out through the trachea (Adams Moss and Fagan 1963) and when this fluid was studied with the dynamic alveolar model it was found to have high surface activity (Enhorning and Adams 1965) A study of the un diluted airway liquid would have been preferable but with the present design of the apparatus it does not permit examinations of the small volumes of liquid ob tainable from the airways of fetal guinea pigs

The observation that the lungs from more mature fetuses yielded a film with greater stabilizing ability is in agreement with previous studies in other species (Avery and Mead 1959, Buckingham and Avery 1962) The close relationship between stability index and phase difference (Table II) should be noted It is conceivable that the increase in both these entities during the 10 min recording is due to an increase in viscosity of the bubble's surface layer Langmuir observed the existence of viscosity in a surface film and described a method for its determination (Langmuir 1917) It was suggested by Avery and Mead (1959) that the surface film of a lung extract has both viscous and elastic entities Adams, Enhorning and Norman (1966) *have discussed these properties further and have come to the conclusion that alveolar* stability may be jeopardized if viscosity of the outlining film is low Recording of the pressure gradient across the surface of a pulsating bubble indicates that, due to visco elastic properties of the outlining film, it resists any change in bubble size When the radius is maintained at a fixed value, the liquid must have a certain negative pressure to overcome the effect of surface tension If the bubble is made larger by withdrawing some of the liquid surrounding it, the pressure in the liquid becomes more negative since the tension in the film increases with stretching When the bubble is made smaller, the surface film resists this change also and the pressure in the liquid surrounding the bubble becomes less negative If the viscosity of the sur face film is high, the film will yield very slowly to the stress it is subjected to during pulsation The most negative pressure will then coincide with the largest size of the bubble and the least negative pressure with the smallest bubble size However, the lower the viscosity of the surface film, the faster it will yield to a stress The change of bubble size during pulsation is sinusoidal Thus as the bubble approaches a max imum or a minimum, the rate of change in surface area becomes slower With low viscosity there will then be time for the surface film to relax prior to maximal stretch ing or compression However, the higher the viscosity of the surface film the greater is the difference in phase and also the pressure amplitude and thus the stability index will be greater Hence the important property of the film lining the alveolus is not limited to its ability to reduce surface tension It may be of equal importance to alveolar stability that the film has the correct visco-elasticity which develops rapidly after initial aeration

The present findings indicate that extracts of immature and mature fetuses can be differentiated by a 2 min pressure recording With the airway washings which were greatly diluted, this was not possible However, the undiluted upper airway fluid of normal newborns may be different from that of infants who subsequently

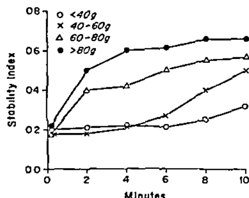


Fig 2 Change of stability index with time Mean values for fetuses in different weight groups Already after 2 min the index was significantly higher for fetuses weighing more than 60 g than for those weighing less

TABLE II Stability index and phase difference two minutes after bubble formation in lung extracts

Group no	Fetal weight	n of subjects	$\bar{S}$ (mean ± S D)	Phase difference in degrees (mean ± S D)
1	<40 g	8	0 21 ± 0 04	125 ± 4 6
2	40—60 g	9	0 18 ± 0 07	120 ± 6 2
3	60—80 g	9	0 40 ± 0 15	150 ± 16 5
4	>80 g	12	0 45 ± 0 15	150 ± 13 2
Difference between groups 2 and 3 (D ± S E)			0 22 ± 0 07	30 ± 8

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Acta physiol. scand. 1966. 68. 43 — 47

From the Institute of Physiology, University of Lund, Sweden

Body Protein and Fat, Blood Glucose and Free Fatty Acids in Various Strains of Mice

By

STIG LARSSON

Received 25 November 1965

Abstract

LARSSON, S *Body protein and fat, blood glucose and free fatty acids in various strains of mice* Acta physiol scand 1966 *68* 43—47

Carcass composition was determined in the NMRI, CBA Swisse and DBA strains and in NMRI × Swisse made obese by injection of goldthioglucose Fasting for 24 hrs resulted in different degrees of weight loss depending on the strain At the same time analysis of carcass composition revealed strain differences with regard to protein and fat The changes of glucose and FFA in plasma after 24 hrs without food also varied among the strains

Food intake is considered to be regulated by the central nervous system, *viz* the diencephalon By lesions in the ventromedial parts of the hypothalamus, the satiety part of the feeding centre is destroyed which leads to an increase in food intake, eventually resulting in obesity (Brobeck 1955) For the normal regulation of food intake these parts of the diencephalon need to be intact On the other hand, spontaneous obesity can occur without any detectable damage of the central nervous system (cf Mayer 1963) The etiology of some of these types of obesity has been intensively studied and partly elucidated In certain types of adipositas, overweight is not found but a relative increase of body fat with age Such data have been given for animals (Moulton 1923) and men (Brozek 1952) Fenton and Dowling (1953) have studied this problem in mice and found that the excessive carcass fat deposition was accelerated by giving the mice a diet high in fat In previous studies in pigs (Larsson, Nilsson and Olsson 1965 and in rats (Larsson 1965) the biological value of the protein of the diet was found to be of great importance for the fat deposition of the body and suggested to exert some influence with regard to obesity The present experiments were undertaken to study the problems related to obesity in mice in relation to different strains of mice viz the carcass composition blood glucose and free fatty acids (FFA)

TABLE I The effect of 24 hrs fasting on body weight in different strains of mice

n = 18 except for NMRI × Swisse where n = 5

Strain	Decrease in weight % of prestarving wt
NMRI	12.2 (10.1–14.2)
CBA	13.7 (12.2–15.6)
Swisse	21.3 (17.9–24.1)
DBA	16.6 (14.3–18.7)
NMRI × Swisse (obese)	15.1 (13.2–18.3)

Experimental

Male mice of the NMRI CBA, Swisse, and DBA strains were used They were all fed *ad libitum* on a fortified laboratory diet used for breeding (AB Teknosan, Sweden) which contained large amounts of animal proteins and about 3 % fat. The protein content was 22.6%. All animals had free access to tap water

Experiment 1

Eighteen mice of each strain were given the diet for 90 days At this time they were all starved for 24 hrs and the change of body weight during the starvation period measured

At the same time 18 other mice of the different strains were kept under the same conditions except that they were not starved on the 90th day

All mice were sacrificed on the 91st day and the bodies of each mouse subjected to individual carcass analysis as has beed described earlier (Larsson 1965) Thus values for total body protein and fat were obtained.

Experiment 2

Ten obese mice were obtained by injecting goldthioglucose (1.2 mg g) Scheering Germany into 70 mice NMRI × Swisse after 24 hrs starving At the time of injection the animals were 65—80 days old. The animals were not used until they had reached a constant weight level on the particular

Experiment 1

Experiment 3

On 20 animals of each of the different strains, blood samples were taken at 85 days of age From here on samples were taken once a week either in the fed or starved state The blood was analyzed as described above with regard to glucose and FFA. In order to obtain enough blood for the FFA determinations, samples from 4 animals were pooled.

Results

Experiment 1

As may be seen from Table I the weight loss after 24 hrs without food was different depending on the strain of mice

The Swisse mice showed the most marked weight loss Table II gives the carcass contents of protein and fat of the mice from the different strains The total protein varies comparatively little in the fed state while the starved animals show definite strain differences Thus the NMRI strain show no relative decrease in body protein after 24 hrs without food The Swisse mice have a pronounced decrease in this respect. Starving decreases fat content in all strain but to different degrees Table

TABLE II The effect of 24 hrs fasting on carcass contents of protein and fat in different strains of mice

F = fed mice S = standard mice n = 18 except for NMRI × Swisse, where n = 5
Differences calculated according to Student's t test

Strain		Protein % of body wt	Fat % of dry body wt	Prot/Fat
NMRI	F	19 1 ± 0 46	32 3 ± 0 98	1 8
	S	20 1 ± 0 43	26 1 ± 0 73[3]	2 3
CBA	F	17 3 ± 0 82	36 9 ± 0 82	1 2
	S	15 2 ± 1 23	22 9 ± 0 91	1 9
Swisse	F	18.8 ± 0 72	23 0 ± 1 10	2 7
	S	13 1 ± 1 12[2]	12 7 ± 0 83	3 4
DBA	F	19 3 ± 1 21	29 6 ± 0 88	2 7
	S	16 6 ± 1 33	15 6 ± 0 90[1]	2 4
NMRI × Swisse (obese)	F	13 4 ± 0 78	68 5 ± 1 63	0 3
	S	13 3 ± 1 26	61 1 ± 1 13[1]	0 5

[1] $P<0.001$, [2] $P<0.01$, [3] $P<0.05$

TABLE III The effect of 24 hrs fasting on plasma content of glucose and FFA in different strains of mice

Glucose n = 20 (obese = 10) FFA n = 5 repeated at 4 occasions (obese = 10 repeated at 4 occasions) Differences calculated as in Table II

Strain	Decrease in blood glucose %	FFA in plasma μeq/ml	
		Fed	Starved
NMRI	57	300 ± 15	350 ± 10
CBA	58	310 ± 14	400 ± 32[3]
Swisse	70	220 ± 24	400 ± 33[1]
DBA	30	460 ± 43	430 ± 26
NMRI × Swisse (obese)	51	280 ± 35	330 ± 33

II also shows the ratio of total body protein and total body fat indicating marked strain differences

Experiment 2

As shown by Table I the obese mice have a 15 per cent decrease in weight after 24 hrs without food The carcass contents of protein and fat in the obese mice as well as the protein — fat ratio may be seen in Table II

Table III gives the values of glucose and FFA in the bloo

Experiment 3

Table III shows that the strains of mice respond differently to the starving period as judged by blood glucose and FFA values

Discussion

The present results indicate that the type of strain of mice plays an important role when studying nutritional problems The response of the animals to a given diet with regard to carcass composition thus varies from strain to strain It has previously been shown that strain differences in mice exist concerning fat mobilization, cardiac glycogen and other metabolic parameters (*cf* Fenton 1960) Certain types of obesity is known to be hereditary in mice (*cf* Mayer 1963) and in dogs (Mayer 1963 and Krook, Larsson and Rooney 1960) Two types of obesity have been recognized namely regulatory due to dysfunction of the hunger-satiety mechanism and metabolic due to a peripheral disorder in metabolism (*cf* Mayer 1963)

The present results indicate that on a given diet the strains of mice studied differed with regard to carcass composition (Table II) Thus, in the fed state the CBA strain deposited more fat than the other strains When fasted for 24 hours, which in reality means a starvation in this species due to high basal metabolic rate, all strains decreased in weight The amount of carcass fat that disappeared during the 24 hrs varied, however The results indicate that the Swisse mice, in the fed state rather "slim", lost more fat relatively during these 24 hrs than did the CBA and NMRI strains This suggests a higher fat mobilizing capacity of the Swisse mice as compared to the other strains studied In the Swisse mice the epididymal fat pad disappeared almost entirely after 24 hrs of fasting As may be seen in Table III the blood sugar at the same time went down to a hypoglycemic value It was also found that 24 hrs without food was critical with regard to the FFA values of the blood A fasting period longer than 24 hours significantly lowered the blood FFA in the Swisse mice whereas 16—24 hrs without food gave elevated values compared to those in the fed state

In contrast to the Swisse mice the NMRI and CBA strains did not seem to mobilize fat so readily after starving for 24 hrs This is supported by the finding that these strains showed less elevation of FFA when fasted and a relatively smaller weight loss The obese mice behaved more similar to the NMRI and CBA strains than to the Swisse mice The obese mice used were NMRI x Swisse to combine the two strains The CBA and partly the NMRI mice can be regarded as fat animals At least the CBA strain has been found to be very sensitive to variation of the protein value of the diet, with regard to fat deposition (Munck 1964) Thus, a diet with low biological value will produce obese animals not judged by the weight but by the carcass content of fat The diet used in the present study had a high value of protein both quantitatively and qualitatively Still high contents of carcass fat was found in the CBA and NMRI mice

In many respects the DBA mice did not fit in with the results from the other strains In spite of an appreciable decrease in fat content of the body when starved for 24 hrs, the FFA level of the blood rather decreased The level of FFA in the fed state was

significantly higher in this group than in any of the others High levels of FFA have been related to thyrotoxicosis (cf Wertheimer, Hamosh and Shafrir 1960) Further it seems as if the DBA mice are very sensitive to rapid changes in the environmental temperature Thus it is possible that this strain posesses a hereditary disorder of the thyroid gland or of the anterior pituitary The lack of response in the fasted state with regard to increase of the FFA level might partly be due to a higher capacity of the liver to take up FFA in this strain Normally the clearance of FFA by the liver is considerable (Steinberg 1964)

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complex central integration mechanisms involved normally and eliminating the hazards of adaptation This paper describes an attempt to perform such a study, where some of the results have earlier been briefly reported (Folkow and Rubinstein 1964)

Material and Methods

fixating them to the skull

Acute experiments A group of 20 rats (Sprague Dawley strain) of both sexes ranging in weight from 200 to 300 g were used in acute experiments under nembutal anesthesia for the localization of the hypothalamic area which in the rat integrates the characteristic autonomic pattern of the defence reaction The animals were placed in a stereotaxic instrument designed for using de Groot s stereotaxic atlas of the rat s brain Arterial blood pressure was measured from a cannulated carotid artery with a Statham P23Db transducer respiration was monitored from a side tube on the tracheal cannula with a Statham P23AC transducer Renal intestinal and muscle blood flows were measured by cannulation of the renal mesenteric and femoral (skinned leg) veins respectively conducting the venous outflow through silicon oil filled lucite drop chambers and then returning the blood to the animal via the jugular vein The drops were counted by a photoelectric cell activating an ordinate writer or one of the channels of a Grass Polygraph where all the above mentioned variables were recorded

Chronic experiments Another 40 rats were selected at random for the chronic experiments with repeated hypothalamic stimulations A single stainless steel electrode (0 5 mm bare tip) was stereotaxically implanted in each animal in the area which in the acute experiments induced the typical autonomic defence reaction The occurrence of a blood pressure rise (tail method) and heart rate acceleration during stimulation was checked after the implantation while the rats were still asleep After full recovery from the anesthesia the stimulation induced modifications in spontaneous behaviour were studied on the freely moving rats (attached to the stimulator by long lightweight

All the rats both stimulated and controls were placed in individual cages and their electrodes

(1700 to 0500 hrs) The stimulus intensity was deliberately set below the level needed for evoking

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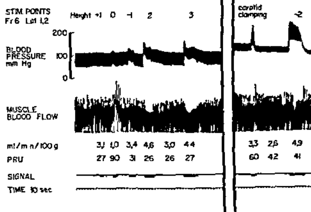


Fig 1 Anesthetized rat Topical hypothalamic stimulation at frontal 6 lateral 1 2 and at different dorsoventral heights, coordinates according to de Groot s atlas As seen from the blood pressure and muscle blood flow recordings a blood pressure rise and a muscle blood flow increase are induced at heights —2 and —3 Atropine 0 3 mg/kg is given between the two panels — Stimulation at the same coordinates elicits in the awake animal increased alertness and ultimately an escape reaction

d ... [illegible]

mean arterial blood pressure in the rat's tail moreover, all the recordings were throughout performed by the same person and the procedure was standardized as far as was possible

Each week mean blood pressure estimations were performed in every animal from both groups until three consecutive comparable readings were obtained for each rat These three recordings from each animal were plotted together with the other data from the animals of the same group where ultimately only six entirely intact rats were left in each group The mean and standard dev ... [illegible]

... Boyd Ltd 1 1938)

Food and water intake of all the animals was measured daily for one week at regular intervals during the course of stimulation All animals were given the same rat food and were kept in identical isolation cages throughout so that the intermittent hypothalamic stimulations in the stimulated group should constitute the only difference from the control group

When the present series of experiments had to be interrupted after 4 months of repeated stimulations gross anatomical studies were conducted on heart major arteries kidneys adrenals and gastrointestinal tract the body weight and the organ weights were measured. The position of the electrode tips was checked with the usual procedure of serial 50 μ frozen sections of the brain fixed with 10 % formaline

Results

1 *Acute experiments* Topical stimulations were performed in anesthetized rats at 0 5 mm steps along the coordinates frontal 6 5 to 5 0 and lateral 0 5 to 1 5 corresponding to different locations in the hypothalamic area Stimulation with 100 imp sec, 1 msec duration and 1 to 3 V at coordinates frontal 6 0 lateral 1 2 and dorsoventral height —2 5 elicited hyperventilation and a characteristic pattern of sympathetic activation — tachycardia, acute hypertension, renal and intestinal vasoconstriction where blood flow decreased despite the raised perfusion pressure, and an increase

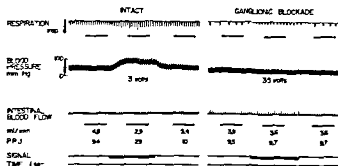


Fig 2 Anesthetized rat Recordings of respiration blood pressure and intestinal blood flow Topical hypothalamic stimulation at frontal 6 the lateral 1.2 and dorsoventral height —2.5 before and after ganglion blockade with Arfonad (Roche) Note the increased respiration blood pressure rise and greatly increased intestinal blood flow resistance where the latter two changes are eliminated by Arfonad.

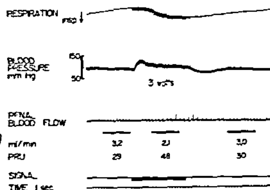


Fig 3 Anesthetized rat Effects on respiration blood pressure and renal blood flow of topical hypothalamic stimulation at the same site as in Fig 2 Note the considerable increase of renal blood flow resistance the blood pressure rise and the increased respiration.

muscle blood flow Fig 1 2 and 3 This response pattern is quite similar to the one observed e g in the cat during defence area stimulation (Folkow and Rubinstein 1965), except for the apparent absence of a cholinergically mediated active vasodilatation in the muscle vascular bed of the rat Thus atropine injection did not affect the vascular response in the skeletal muscles of the rat Other studies may, however, indicate that only some mammals mainly carnivorous animals like dogs, cats, etc , have a really significant supply of sympathetic cholinergic vasodilator fibres (Lynas 1965

II *Chronic experiments* With the electrodes placed at the coordinates mentioned above a group of animals was kept under a schedule of repeated hypothalamic stimulations as described in Methods The spontaneous behaviour of these animals when not actually exposed to a hypothalamic stimulation which always alerted them was apparently in other respects unchanged and similar to that of the control animals, and they kept their normal rhythm of activity

The main technical difficulty was to keep the wires and electrodes from breaking, and such events gradually reduced the number of intact animals in both groups

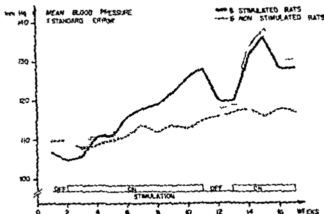


Fig 4 A diagram illustrating the gradual change of the resting mean blood pressure in 6 rats which for a prolonged period were intermittently stimulated in the hypothalamic defence area As a

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to be again rapidly raised when the stimulations are restarted

However a group of 6 stimulated animals and a corresponding number of controls were kept in excellent condition, tolerating well the isolation and restraint The stimulations could here be maintained for about four months except for a period of "rest" of two weeks (between the 11th and 13th) The ultimate results will correspond then to those 6 rats that could be kept under the desired stimulation schedule, compared with the other 6 control animals living under exactly the same conditions, i e isolation restraint, with one implanted electrode in the hypothalamus but no stimulation The combined effect of the restrained living conditions plus the electric hypothalamic stimulations caused no significant modification in the food and water intake of the stimulated animals as compared with the controls Also their body weight increased in a similar fashion as in the controls These findings seem to exclude the possibility of gross accidental stimulations or electrode damages to e g the close by feeding center of the rat which is known to induce a significant increase in the food intake (Smith 1961)

The main findings are summarized in Fig 4 and Table I In Fig 4 is depicted the course of the resting mean blood pressure during the weeks of intermittent hypothalamic stimulations in the group of 6 stimulated rats and in the control rats As is seen from the figure the stimulated rats were throughout kept on the schedule of daily stimulations except for an interval between the 11th and the 13th week The diagram shows a very moderate but gradual increase in resting blood pressure as recorded in the awake rats at least 6 hrs after the end of the period of stimulation of the day The difference in the blood pressure levels started to be significant after the 8th week of daily stimulations The interruption of the schedule for two weeks on the 11th and 13th week caused a significant decrease of the blood

TABLE I The table shows the mean blood pressure levels ± standard error (S E) in the stimulated rats and the non-stimulated control rats during the entire period of experiment

Weeks	Stimulated group mean ± S E 6 rats	Non stimulated group mean ± S E 6 rats	Significance (p less than)
1	107 ± 1 8	110 ± 1 6	N S
2	105 ± 1 9	110 ± 2 0	N S
Stimulation on			
3	106 ± 2 3	108 ± 2.5	N S
4	111 ± 2.2	109 ± 1 9	N S
5	111 ± 2 4	110 ± 2 2	N S
6	116 ± 2 3	111 ± 2 2	N S
7	118 ± 2 5	114 ± 2 4	N S
8	119 ± 2 4	112 ± 2 3	N S
9	122 ± 2 2	114 ± 2 3	0 05
10	126 ± 2 5	113 ± 2 3	0 01
11	128 ± 2 6	115 ± 2 0	0 01
Stimulation interrupted			
12	120 ± 2 1	116 ± 2 2	N S
13	120 ± 2 0	117 ± 2 3	N S
Stimulation restarted			
14	131 ± 2 4	118 ± 2 0	0 01
15	136 ± 2 3	116 ± 2 1	0 01
16	128 ± 2 2	118 ± 2 2	0 02
17	128 ± 2 1	117 ± 1 9	0 02

pressure level in the stimulated rats, so that they approached the pressure levels of the controls When the stimulation was resumed, the level of 'resting blood pressure rose rapidly to the values seen in the stimulated rats just before the interruption, rather than showing the slow pressure increase that occurred when the stimulations were started This new pressure rise was maintained at a significantly higher value as compared with the controls up to the end of the experiment

The gross anatomical studies showed that the organs of the stimulated animals and their body weights were not significantly different from those of the controls In only 2 of the 6 hypertensive rats there was a moderate but hardly significant increase in heart weight No gross vascular changes were observed It should here be stressed, however that the stimulations had deliberately been kept fairly weak so as to create only a moderate intermittent impact on the cardiovascular system

Discussion

The main requirement for this experimental approach was to find a hypothalamic area, from where it would be possible to elicit an autonomic activation corresponding to the defence reaction in other experimental animals and man which evidently is normally activated in most alarm situations and [illegible] of

mental stress This was accomplished, as was mentioned in Results, by the series of acute experiments However, there was no evidence of a cholinergic vasodilatation in the muscle vascular bed of the rat As the engagement of these specific sympathetic fibres in the cat, at least, is considered an almost *sine qua non* component of the defence reaction (Abrahams, Hilton and Zbrozyna 1960), the lack of its finding in the rat may at first sight be somewhat surprising However, as mentioned earlier, it might be so that in mammals a really significant supply of cholinergic sympathetic vasodilator fibres to the skeletal muscles is present only in carnivorous animals (Uvnas 1965) In all other respects the autonomic component of the defence reaction, and its somatomotor counterpart, was closely similar in the rat as compared with the cat

Since the experiments with chronic stimulations met with great technical difficulties they should, with respect to the results based on only the small number of animals ultimately left, be considered as preliminary ones, studies on a larger scale are needed In any case, this type of approach in animal experiments, linked to long term clinical studies in man (cf Charvat, Dell and Folkow 1964), both seem to be necessary for a reliable evaluation of the importance of mental stress for the gradual development of a chronic hypertensive state, as triggered by the centrally induced intermittent autonomic nervous and hormonal discharge which accompanies such situations

This small series of pilot experiments on chronic rats is promising insofar as the results suggest that a gradual shift of the resting blood pressure level really takes place in the stimulated animals This, despite the fact that the topical hypothalamic stimulations in the present experiments were throughout very mild and did not involve any drastic, acute disturbances of either behaviour or the cardiovascular equilibrium The gradually developed, significant shift in the resting blood pressure was, however, not sustained in the rats for long periods of time but approached the pressure level of the controls if the stimulations were interrupted for two weeks When, the stimulations started again, the resting pressure level was soon raised to the level seen just before the two week interruption, rather than showing the gradual rise, seen at the initiation of the stimulation period These findings are of interest for two reasons First, they render more confidence to the differences in pressure levels observed and make it far less likely that this difference was only due to chance Second they suggest that, in rats at least, the resting blood pressure equilibrium will tend to return towards normal once the trigger factor is eliminated at least as long as it is a matter only of a fairly mild degree of induced hypertension The gradual rise in resting blood pressure is certainly not a consequence of some sort of perpetuated hypothalamic after-discharge, induced by the brief periods of topical stimulations, because regularly the acutely induced cardiovascular adjustments disappear well within a minute after ended stimulation

Whether there is any direct correlation between the average peak increase in pressure during the intermittent stimulations and the rate and extent of the gradual increase of resting blood pressure level is not known at present Hov[illegible]

being equal, it seems likely that the more the average blood pressure load is increased during a prolonged period, the more extensive secondary cardiovascular changes will become (cf Folkow 1960) It should, on the other hand, be realized that also other factors, besides the neurogenically induced load on the cardiovascular system, are involved in the defence reaction For instance, topical activations of the defence area have been shown to trigger a profound release of ACTH, corticoids (Folkow, Hedner, Lisander and Rubinstein 1965), and also catechol amines, mainly adrenaline (Grant *et al* 1958) It is, in addition likely that an increased aldosterone secretion is involved This means that the impact of mental stress on the cardiovascular system should not only be considered as mediated via the autonomic nervous system *per se*, acting on the cardiovascular effectors directly It will quite likely also involve in a most important way some more indirectly acting hormonal influences, which affect the cardiovascular system by changing aspects like the water-salt balance, thus affecting the blood volume, or the ionic equilibrium across the membranes of cardiovascular effectors, etc, which may considerably affect their "reactivity" (Charvat, Dell and Folkow 1964) Thus, what may at first sight appear to constitute a purely neurogenic trigger factor, elicited from higher nervous centers in a way that mimicks the events taking place during mental stress, is far from purely neurogenic but rather should be considered as a complex of centrally elicited neuro hormonal factors which in both direct and indirect ways affect the cardiovascular system

It is not surprising that no gross morphological changes could be observed in the stimulated animals After all, they had deliberately been exposed to only fairly weak excitations of the defence area and the gradual, secondary rise in resting blood pressure was so moderate that it can hardly be expected to have led to more obvious morphological changes in vessels or heart It is possible that the situation would, in this respect have been different in case more intense stimulations had been performed This would on the other hand, have greatly increased the risks of damaging the stimulation wires and electrodes Further, other hypothalamic areas, controlling e g food and water intake, temperature regulation etc might then also have been affected, causing gross disturbances of the animals general homeostasis, a situation to be avoided in a project of this nature

This study was supported by grants from the World Health Organization Medical Research Grant R/00038 from Air Force School of Aerospace Medicine under Contract AF 61 (052) 739 through the European Office of Aerospace Research OAR United States Air Force from U S Public Health Service (Grant HE-05675 03-01 and by a grant from the Swedish Medical Research Council

AB Hassle Goteborg Sweden has generously covered the employment of a technician

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Acta physiol scand 1958 *43* 135—154

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Acta physiol scand 1966 68 58 — 63

From the Institute of Physiology and Medical Biophysics University of Uppsala Sweden

A Method for Intracardiac Recording of Monophasic Action Potentials in the Dog Heart in Situ

By

ULF SJOSTRAND

Received 20 January 1966

Abstract

SJOSTRAND, U *A method for intracardiac recording of monophasic action potentials in the dog heart in situ* Acta physiol scand 1966 *68* 58—63

and duration of the action potential

'An understanding of electrocardiographic complexes will be achieved when the potential at a given body surface point can be predicted from a knowledge of ventricular depolarization and repolarization pathways To do this for QRS we must have exact information on three factors First, we must know the time course and magnitude of potential changes across the membranes of the ventricular syncytium as depolarization takes place Second we must know the pathway of ventricular depolarization Third we must understand the basic principles of current flow in volume conductors and the modification of these principles necessitated by the resistive inhomogeneity of the tissues and the irregular shape of the body

This citation is taken from a paper of Scher and Young published in the Annals of the New York Academy of Sciences 1957 and reflects what from a theoretical aspect is required for understanding of the electrocardiogram

Regarded from a practical clinical aspect the main importance of electrocardiography lies in the fact that it gives in a relatively simple way essential information on the excitation rhythmicity and impulse propagational conditions in the heart By investigations on animals the appearance of the action potential in different regions of the heart has been studied in vitro and the impulse propagation has been studied in vivo The information concerning excitation and propagation which is

obtainable from the potential changes recorded on the body surface, i e the electrocardiogram (ECG), applies to large and as a rule not absolutely definable sections of the conducting system and the atrial or ventricular muscle For technical reasons (volume conductor recording) it is not possible with electrodes applied to the body surface to record the activity of the SA node or the electrical activity in other parts of the conducting system (e g the AV node or the bundle of His) for each individual cardiac cycle, analogically neither is it possible with external electrodes to record and localize the electrophysiological activity during the individual cardiac cycle in small anatomically defined regions of the atrial and venticular muscle Area Display Electrocardiography (Schuler and Roy 1965) appears, however, to give a spatial picture of the electrophysiological mean activity in relation to the ECG in relatively limited regions of the myocardium

The size and course of the action potential in the human heart is only partially known Only two studies of the transmembrane action potential in human atrium muscle (in vitro) have been published (Trautwein *et al* 1962, Sleator and de Gubareff 1964), whereas the action potential in the different cardiac regions in both warm- and cold blooded animals has been relatively well investigated (for review see Weidmann 1956 a, Weidmann 1957) The path by which the impulse is propagated has been studied experimentally in the dog (for review, see Durrer *et al* 1965, Scher 1965), in different types of monkeys (Scher 1965) and in man (Durrer *et al* 1965) With regard to the distribution of the electrical activity of the heart out to the body surface, there is a good review by Gelernter, Swihart and Angell (1965)

A technique for intracardiac recording of action potentials in the intact mammal heart (including the human heart) should make it possible to study the subendocardially situated conducting system in both the atrium and the ventricle, and also to record the action potential in subendocardial atrial and ventricular muscle The form and duration of the intracardially recorded monphasic action potential, and also its time relationship to the simultaneously recorded ECG, should provide information of the electrophysiological conditions at the recording point, thereby enabling a detailed analysis to be made in demarcated sections of the cardiac chambers By roentgenological localization of the position of the recording point (recording of intracavitary ECG provides further possibilities for localization — especially as regards pacemaker regions) it should be possible with such a method to determine the normal conditions of impulse propagation both in man and lower animals and also the impulse formation and propagation in cases with a pathological ECG Principal prerequisites for arhythmia which have been established on the basis of experimental action potential studies in animals could by this means also be evaluated directly in the intact human heart It should further be possible to study the effect of intracardiac pressure changes Teorell 1965 drugs for review see Traut wein 1963), electrolytes and blood gas composition on impulse formation and impulse propagation in the heart Furthermore by this method it should be possible during open cardiac surgery to localize conducting structures so as to avoid deleterious traumatic damage during the surgical procedure

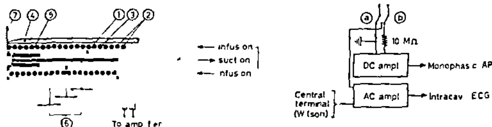


Fig 1a (left) The intracardiac end of the suction electrode equipment lying against the endocardiac surface (semi schematic) 1 = polyethylene tube 2 = stainless steel wires one central and adjustable the other attached to the stainless steel tube at the tip 3 = stainless steel coil spring 4 = X ray opaque catheter 5 = stainless steel tube 6 — indication of adjustment possibilities relative to X ray opaque catheter 7 = endocardiac surface By means of the switch shown in Fig 1b alternation can be made between electrogram — monphasic AP (position a) and intracavitary ECG (position b, AC-ampl — one of the alternating current amplifiers in the ECG writer)
Fig 1b (right) Block diagram of the amplifiers and registration systems *Cf* legend of Fig 1a

Method

The intracardiac electrical activity (electrogram and monophasic action potential respectively) was recorded by means of a suction electrode technique (see e g Sjostrand 1964) The suction electrode was of such dimensions and form that it could be passed through conventional heart catheters for clinical use Fig 1a shows the part of the suction electrode equipment that was applied intracardially The suction electrode consisted in this case of a polyethylene tube (Fig 1a 1) 800 mm long and with an inner diameter of 0 8 mm A stainless steel wire (Fig 1a 2) with a diameter of 0 25 mm ran through the centre of the polyethylene tube The polyethylene tube with the central stainless steel wire was then introduced into a coil spring of stainless steel wire (Fig 1a 3 diameter of wire 0 3 mm inner diameter of coil spring 1 mm) The stainless steel coil spring with its contents can be passed into an ordinary X ray opaque plastic catheter (Fig 1a 4 Ödman Ledin type grey Kifa Stockholm Sweden inner diameter 1 8 mm) intended for cardiac catheterization In order to obtain better recording conditions a stainless steel tube (Fig 1a 5) was introduced into the end of the polyethylene tube and to this stainless steel tube was attached a 0 1 mm stainless steel wire (Fig 1a 2) which ran through the centre of the polyethylene catheter As may be seen in Fig 1a the mutual positions of the central stainless steel wire the polyethylene tube the stainless steel coil spring and the X ray opaque cardiac catheter could be regulated

1964) conditions are created at the site of suction for good contact between the electrode and the protoplasma of the underlying cells (in order that any KCl leaking out shall not reach a high concentration outside the central suction catheter this is washed out with a solution with a normal KCl concentration

Sjostrand 1966) recording is made with a Tektronix oscilloscope (type 561 Tektronix Inc Portland U S A) and also parallel with this in one channel (direct current ampl fication) of a four channel Elema Mingograph (type 42 B Elema Schonander AB Stockholm Sweden) in experiments on the dog the remaining three channels record the standard leads (subcutaneous electrodes) The frequency characteristics of the Mingograph do not permit a true recording of the form of the action potential, the recorder was accepted however since the time relation of the action potential was of interest — As shown in Fig 1b intracavitary ECG can also be recorded in the usual way with the coil spring as the detecting and a central terminal (Wilson is the other electrode

The suction which is appl ed is about 600 mm Hg The central suction electrode is filled with isotonic electrolyte solution in which part of the NaCl has been replaced with KCl so that the K concentration is 44 mM

Experiments were performed both on toads (*Bufo bufo*) which had been decapitated and on dogs which had been anaesthetized with a combination of chloralose and Nembutal® In frogs the

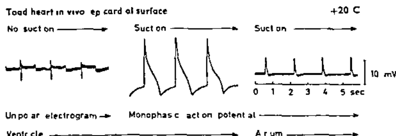


Fig 2 Three recordings from the same toad heart (in vivo) where the tip of the suction electrode equipment (cf Fig 1a) is placed against the epicardiac surface (Elema Mingograph 42 B)

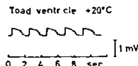


Fig 3 Recording from toad heart (epicardiac surface ventricle) with the suction electrode equipment mentioned on p 5 (experiments on man)

suction electrode equipment was applied to the exposed epicardiac atrial or ventricular surface — these experiments were performed in order to ascertain by an inexpensive and easy method some of the principal characteristics of the recording electrode equipment In the dog the equipment was introduced via the right femoral vein and the tip of the catheter was placed in position by direct intrathoracic manual means via a right sided intercostal (about 8 cm long) thoracotomy (for this the dog was ventilated at excess pressure)

Results

Fig 2 shows an experiment on the toad On placing the electrode against the ventricular surface a ventricular electrogram is recorded, which on suction and slight penetration of the central stainless steel wire is transformed to a monophasic action potential A similar but somewhat smaller action potential is also obtained, from the electrode when applied in the same way in the atrium Fig 3 shows a recording from the ventricular surface in another toad, in which a better plateau was obtained (with regard to suction electrode recordings from the sinus venosus and atrium in the toad reference may be made to Sjostrand 1964)

Fig 4 shows ECG (standard leads) and action potential recordings in dogs In this case (a) the suction electrode was applied in or in the vicinity of the sinus node region, and as may be seen in the figure the recorded action potential has a depolarization phase appearing before the P wave in the electrocardiogram on recording with the four channel writer (same time scale for all recordings) The figure also shows a recording (b) with the suction electrode applied in the apical part of the right ventricle, and it may be seen that the depolarization phase of the recorded action potential coincides with the QRS complex which is in complete agreement with what is known of the impulse propagation in the dog heart and what has also been accepted for the human heart (cf Goldman 1964) Fig 5 shows an oscilloscopic recording of the same section as Fig 4 b)

Discussion

With regard to the relationship between the action potential recorded by the micro-electrode technique and that recorded by the suction electrod

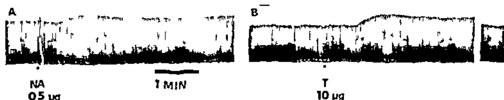


Fig 1 Perfusion of isolated heart of *Lampetra fluviatilis*
A Effect of noradrenaline (NA) 0 5 µg
B Effect of tyramine (T) 10 µg

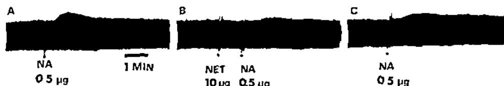


Fig 2 Perfusion of isolated heart of *Lampetra fluviatilis*
A Effect of noradrenaline (NA) 0 5 µg
B Effect of noradrenaline (NA) 0 5 µg 1 min after injection of Nethalide (NET) 10 µg
C Effect of noradrenaline (NA) 0 5 µg 25 min after the injection of Nethalide 10 µg

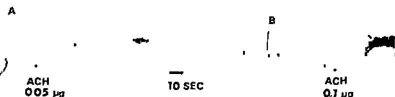


Fig 3 Perfusion of isolated heart of *Lampetra fluviatilis*
A Effect of acetylcholine (ACH) 0 05 µg
B Effect of acetylcholine (ACH) 0 1 µg

of Augustinsson *et al* (1956) Isoprenaline also produced positive inotropic and chronotropic effects of the same nature as that of adrenaline and noradrenaline The effect of the different agents was in the order isoprenaline > adrenaline > noradrenaline Lowest effective dose of adrenaline was 0 05 µg In hearts sensitive to adrenaline and noradrenaline, tyramine in doses of 10—25 µg caused positive inotropic and chronotropic effects similar to those of adrenaline and noradrenaline (Fig 1)

The positive inotropic and chronotropic effects of adrenaline, noradrenaline and isoprenaline were readily blocked by Nethalide (Fig 2), a β-receptor blocking agent (Black and Stephensson 1962)

Acetylcholine and nicotine were reported by Augustinsson *et al* (1956) to produce a negative inotropic and a positive chronotropic effect in the isolated lamprey heart These authors also reported an inhibition of the effect of acetylcholine by curare These observations were verified in the present investigation In some hearts acetylcholine was effective in doses of 0 05—0 1 µg (Fig 3) and in these more sensitive hearts 10 µg of nicotine produced an acetylcholine-like effect The response

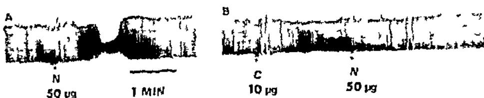


Fig 4 Perfusion of isolated heart of *Lampetra fluviatilis*
A Effect of nicotine (N) 50 µg
B Effect of nicotine (N) 50 µg after pretreatment with curare (C) 10 µg

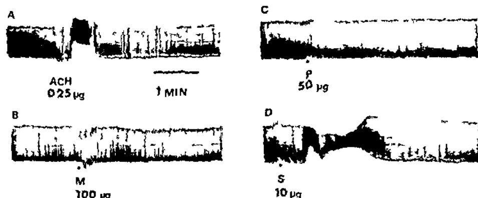


Fig 5 Perfusion of isolated heart of *Lampetra fluviatilis*
A Effect of acetylcholine (ACH) 0 25 µg
B Effect of dl muscarine (M) 100 µg
C Effect of pilocarpine (P) 50 µg
D Effect of succinylcholine (S) 10 µg

to acetylcholine was not influenced by atropine nor was the effect of nicotine inhibited by hexamethonium but the effects of both acetylcholine and nicotine could, however, be blocked by curare (Fig 4) Further succinylcholine in doses of 10 µg produced an effect similar to that of acetylcholine and nicotine (Fig 5) The effect of succinylcholine was also inhibited by curare but not by atropine dl Muscarine and pilocarpine were tested in doses up to 100 µg without altering the rate and contractile force of the lamprey Three out of the 20 hearts were insensitive to acetylcholine One of these belonged to the group which was insensitive to the catecholamines and tyramine

B Pleuronectes platessa

Adrenaline noradrenaline and isoprenaline produced positive inotropic and chronotropic effects on the isolated heart of the plaice The effect of these agents was in the order isoprenaline > adrenaline > noradrenaline, isoprenaline being effective in doses of 0 05–0 1 µg The responses to adrenaline, noradrenaline and isoprenaline were blocked by Nethalide

Tyramine injected in doses up to and including 0 1 mg was ineff
Higher doses were not tested

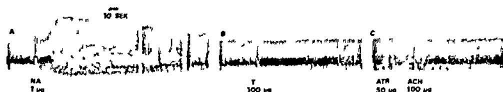


Fig 6 Perfusion of isolated heart of *Pleuronectes platessa*
A Effect of noradrenaline (NA) 1 µg
B Effect of tyramine (T) 100 µg
C Effect of acetylcholine (ACH) 100 µg after pretreatment with atropine (ATR) 50 µg

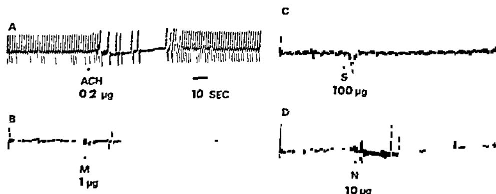


Fig 7 Perfusion of isolated heart of *Pleuronectes platessa*
A Effect of acetylcholine (ACH) 0 2 µg
B Effect of dl muscarine (M) 1 µg
C Effect of succinylcholine (S) 100 µg
D Effect of nicotine (N) 10 µg

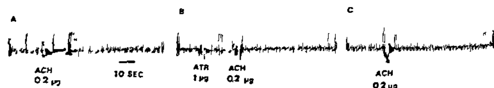


Fig 8 Perfusion of isolated heart of *Pleuronectes platessa*
A Effect of acetylcholine (ACH) 0 2 µg
B Effect of acetylcholine (ACH) 0 2 µg after pretreatment with atropine (ATR) 1 µg
C Effect of acetylcholine (ACH) 0 2 µg 15 min after the injection of atropine 1 µg

Acetylcholine in doses of 0 1 – 0 2 µg and dl muscarine in a dose of 0 5 µg produced negative inotropic and chronotropic effects (Fig 7) which could be blocked by atropine (Fig 8) Succinylcholine in doses up to 0 1 mg was without effect (Fig 7) Nicotine in a dose of 10 µg produced negative inotropic and chronotropic effects of the same nature as that of acetylcholine and muscarine (Fig 7) and the effect of nicotine was blocked by hexamethonium (Fig 9) When two injections of nicotine were given in rapid succession the heart became insensitive to this second dose After 10—15 min rest the response to nicotine returned to normal again

A survey of the different agents tested and their effects on the isolated hearts of the two species is given in Table I and II

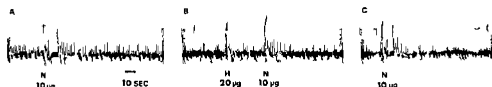


Fig 9 Perfusion of isolated heart of *Pleuronectes platessa*
A Effect of nicotine (N) 10 μg
B Effect of nicotine (N) 10 μg after pretreatment with hexamethonium (H) 20 μg
C Effect of nicotine (N) 10 μg 15 min after the injection of hexamethonium 20 μg

TABLE I Perfusion of isolated heart of *Lampetra fluviatilis* Survey of the effect of different agonists and corresponding antagonists

Agent tested (Agonists)	Effect	Effect inhibited by (Antagonists)
Acetylcholine	Neg inotr and pos chronotr	d tubocurarine
dl Muscarine	0	
Pilocarpine	0	
Succinylcholine	Neg inotr and pos chronotr	d tubocurarine
Nicotine	Neg inotr and pos chronotr	d tubocurarine
Adrenaline	Pos inotr and chronotr	Nethalide
Noradrenaline	Pos inotr and chronotr	Nethalide
Isoprenaline	Pos inotr and chronotr	Nethalide
Tyramine	Pos inotr and chronotr	Nethalide

TABLE II Perfusion of isolated heart of *Pleuronectes platessa* Survey of the effect of different agonists and corresponding antagonists

Agent tested (Agonists)	Effect	Effect inhibited by (Antagonists)
Acetylcholine	Neg inotr and chronotr	Atropine
dl Muscarine	Neg inotr and chronotr	Atropine
Pilocarpine	Neg inotr and chronotr	Atropine
Succinylcholine	0	
Nicotine	Neg inotr and chronotr	Hexamethonium
Adrenaline	Pos inotr and chronotr	Nethalide
Noradrenaline	Pos inotr and chronotr	Nethalide
Isoprenaline	Pos inotr and chronotr	Nethalide
Tyramine	0	

Discussion

The variation in sensitivity of the lamprey hearts to the test agents is not understood It could neither be correlated to sex or age or the period of time the animals had been stored before use nor to any histochemically detectable changes in the

catecholamine stores. Hearts which were sensitive to adrenaline and noradrenaline could be insensitive to acetylcholine and vice versa but most hearts, however, responded to adrenaline, noradrenaline and acetylcholine. Nevertheless there was one heart which was unresponsive to all these agents.

As stated previously, hearts of the lamprey, which were insensitive to noradrenaline and adrenaline were also insensitive to tyramine. Otherwise tyramine caused positive inotropic and chronotropic effects. On the other hand, hearts of the plaice were insensitive to tyramine, nor did large doses of acetylcholine produce any sympathomimetic effect after atropine.

It is well known that in organs where catecholamines have been depleted by reserpine treatment, tyramine is without effect but on treatment with noradrenaline or one of its precursors the effect of tyramine is restored (for references see Andén *et al.* 1964). While the effect of tyramine on the isolated lamprey heart may be explained by the release of existent stores of catecholamines (Dahl *et al.* 1966), its lack of effect on the plaice heart may be due to the absence of stores of that kind (von Mecklenburg 1966). Since no adrenergic fibres exist in any of these hearts the catecholamines released by tyramine in the lamprey heart must come from the catecholamine containing cells. Also, on the isolated hearts of both the lamprey and the plaice adrenaline, noradrenaline and isoprenaline produced positive inotropic and chronotropic effects which are readily blocked by a β receptor blocking agent (Nethalide). It would thus appear that the adrenergic receptors in these hearts are of the same nature as those in the mammalian heart, i.e. of the β type according to Ahlquist's definition (Ahlquist 1948, 1962).

In the isolated mammalian heart, nicotine produces a biphasic response, initially negative inotropic and chronotropic effects followed by positive inotropic and chronotropic effects. Both responses are blocked by ganglionic blocking agents (Kottegoda 1953). Only the initial response is blocked by atropine indicating that this part of the nicotine effect is due to the stimulation of cholinergic receptors. The second response of this drug is blocked by β receptor blocking agents, and should thus represent the stimulation of adrenergic receptors. According to Burn and Rand (1958) the positive inotropic and chronotropic responses are due to the release of catecholamines from storage sites in the heart.

In the isolated lamprey and plaice hearts nicotine does not produce any biphasic response. In the former, nicotine causes a positive chronotropic and a negative inotropic effect which are unaffected by hexamethonium. Acetylcholine and succinylcholine produce effects similar to that of nicotine which are not antagonized by atropine. The effects of all three, nicotine, acetylcholine and succinylcholine are, however, blocked by curare. Since pilocarpine and muscarine are also ineffective in this preparation, the only existing cholinergic receptor in this heart seems to be of the curare sensitive type. After atropinisation of the mammalian heart, larger doses of acetylcholine produce positive inotropic and chronotropic effects due to the release of catecholamines (for references see Burn and Rand 1965). In the lamprey heart, larger doses of acetylcholine do not produce any sympathomimetic effects

after pretreatment with curare (Fig 6) Thus the catecholamines in the heart of the lamprey are not released by nicotine or acetylcholine

In the isolated plaice heart nicotine produces negative inotropic and chronotropic effects of the same nature as that of acetylcholine and which are readily blocked by hexamethonium and atropine A negative inotropic and chronotropic effect is also produced by muscarine and the effect of this agent is likewise blocked by atropine

The cholinergic receptors in the plaice heart thus appear to be similar to those in the mammalian heart i e of the hexamethonium and the atropine sensitive type

The research reported in this paper has been sponsored by the Air Force Office of Scientific Research under grant AF EOAR 66—14 through the European Office of Aerospace Research (OAR) United States Air Force and by the Swedish Natural Science Research Council (99—32)

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Acta physiol. scand. 1966. 68. 72 — 83

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Some Properties of the Thalamic Relay Cells in the Spino-Cervico-Lemniscal Path

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Received 1 February 1966

Abstract

Andersen, P , S A Andersson and S Landgren *Some properties of the thalamic relay cells in the spino-cervico lemniscal path* Acta physiol scand 1966 *68* 72—83

Cells in *nucleus ventralis posterolateralis thalami* lateral to the relay cells of the hindlimb component of e dorsal column pathway have been studied with extra and intracellular recordings in cats tized with pentobarbital sodium The cells in this part of thalamus are characterized by a

se

be antidromically invaded from the ipsilateral cortical areas S I and S II A number of cells were antidromically invaded from both S I and S II indicating that these cells project to cortex with a divided axon Many cells showed an inhibition of the recurrent type

The thalamic relay of the spino cervico lemniscal tract (SCLT) is located in a border zone laterally in the *n ventralis posterolateralis* (VPL) In this region, convergence is observed between this path the dorsal column lemniscal path (DC) from the hindlimb, and skin afferents from the forelimb (cf Landgren, Nordwall and Wengstrom 1965) The response evoked from the SCLT has a shorter latency and is usually larger in amplitude than the response evoked from the dorsal column path (DC) or from the forelimb In order to explain the great efficiency of excitation via the SCLT it was considered desirable to study the excitatory events in its thalamic relay station by intracellular recording

The SCLT is known to have a cortical projection to S I and S II (Morin 1955, Norrsell and Voorhoeve 1962, Andersson 1962) Therefore, the cortical connections of the cells in the border zone were studied by antidromic activation from different cortical points Such a study is of particular importance for cells with convergence from different peripheral sources since in the posterolateral part of the ventral nucleus

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(VPL) such cells have been assumed to be interneurones (Andersen, Eccles and Sears 1964 b)

In this report evidence will be presented indicating a thalamo-cortical projection from thalamic cells activated from SCLT only, as well as from cells receiving afferent excitation from several peripheral sources It will also be shown that cells in this thalamic region have bifurcating axons projecting to two different cortical regions, located in S I and S II respectively Evidence concerning inhibitory interaction between the afferent paths to the cells in the border zone will also be presented These findings indicate that these cells are subject to a similar type of recurrent inhibition as demonstrated for neurones in the main part of the VPL by Andersen, Brooks, Eccles and Sears (1964 a)

Methods

[illegible]

overlying fimbria and hippocampus

The head of the animal was fixed in a Horsley Clarke holder and the microelectrode was inserted into the thalamus according to Horsley Clarke co-ordinates

The brain was covered with a pool of paraffin oil at 37° C. The cortical potentials evoked by elec- [illegible]

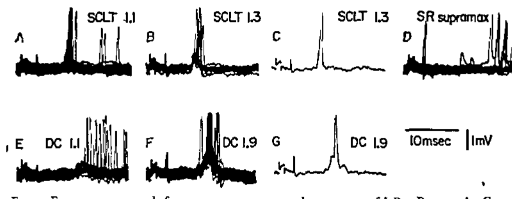
(Grass 4 S) and the stimulating current measured by a current probe (Hewlett Packard 465 A)

The contralateral superficial radial median and ulnar nerves were dissected for electrical stimulation with bipolar electrodes The spinal cord was exposed in the low thoracic region The dorsal columns and the contralateral dorsolateral funiculus were cut and dissected for stimulation in an ascending direction by bipolar electrodes The forelimb nerves and the spinal fascicles were covered by warm paraffin in pools made from the skin flaps

The recording electrodes were glass micro-pipettes filled with solutions of either 4M NaCl 3M KCl or 2M potassium citrate having resistances of 2—10 MΩ The signals obtained from the micro electrode were fed into a cathode follower circuit which was able to neutralize the capacity between the electrode and ground To unblock the microelectrode the neutralizing amplifier could be brought into oscillation The cathode follower also delivered a square wave pulse that indicated the resistance of the microelectrode The neural activity was displayed both on an oscilloscope via an AC coupled preamplifier and on a second oscilloscope with a DC coupled input in order to record the membrane potential

The localization of the spino-cervico-lemniscal border zone in VPL

The spino-cervico-lemniscal border zone is located dorsally laterally and ventrally to the somatotopically organized part of VPL where the dorsal column lemniscal path from the hindlimb evokes a focal potential of maximal amplitude (cf Landgren et al 1965 In the beginning of each experiment a grind of exploring tracks was made using a low resistance NaCl filled microelectrode The electrode was directed according to Horsley Clarke co-ordinates and the landmarks on the dorsal surface of the thalamus were noted After completion of the grid the transversal area of VPL was known in terms of manipulator-co-ordinates The microelectrode was then replaced by a high resistance microelectrode for recording from single neurones The vertical co-ordinates of the new electrode were related to those of the exploring one by means of the haircross of an optical device in a fixed position on the stand carrying the micromanipulator Additional information concerning the location of the recording sites was obtained during each subsequent track by observing the pattern of convergence between the different afferent inputs.



the resistence of the microelectrode

Results

Pattern of convergence

Intracellular recording from cells in the spino cervico lemniscal border zone of *n ventralis postero lateralis* thalami (VPL) is difficult because the cells in this region are small in size (mean diameter 19 μ SD 5 μ) Nevertheless, it was possible to investigate a number of neurones intracellularly under reasonably stable conditions and with resting potentials in the order of 20—60 mV Additional information was obtained from records of extracellular giant spikes Altogether 52 cells in the zone of convergence were investigated 21 cells were excited only from SCLT and the remaining 31 cells were in addition excited from DC and/or forelimb nerves

Fig 1 shows extracellular records from a border zone cell that was excited by electrical stimulation of the SCLT and of the DC at the level of L I, and of the superficial radial nerve (SR) At a stimulus strength of 1 1 times threshold of the thalamic response (T) the SCLT gave an excitatory postsynaptic potential (EPSP) in most of the trials The cell discharged on the summit of the EPSP with the exception of a few action potentials which appeared with a latency that was about twice as long (Fig 1 A) When the stimulus strength was increased to 1 3 T (B) the EPSP appeared in every sweep and the action potentials all had a rather constant latency

Stimulation of the DC at a strength of 1 1 T (E) gave an EPSP with a longer latency The action potentials appeared asynchronously on the falling phase of the extracellularly recorded EPSP With a stimulus strength of 1 9 T (F) the action potential appeared earlier on the EPSP and the response was divided into two peaks The duration of the EPSP evoked from the SCLT was much shorter than that produced by the DC as can be seen by comparing records A and F The single records in C and G show the difference in the rising phase of the EPSP evoked from stimulation of the SCLT and the DC The SCLT gave a steep rising phase and the action potential was elicited on the crest with very little delay The DC gave a slower

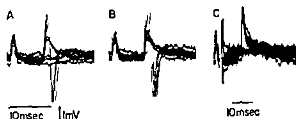


Fig 2 Intracellular records from a cell excited only from SCLT Record A obtained at threshold strength and B at a slightly higher intensity of the stimulus show the unitary character of the synaptic action of the SCLT volley In record C the threshold strength stimulus to the SCLT was preceded by an electrical stimulus to the cortical area S II giving antidromic invasion of the cell Membrane potential 30 mV

and irregular rising phase of the EPSP and the action potential appeared on the falling phase with a considerable delay The small hump that appears after the action potential in record G probably corresponds to the second group of action potentials in record F The cell was also discharged by stimulation of SR but with a longer latency (D) and sometimes with multiple spikes The EPSP is obviously polysynaptically produced The unitary EPSP with shorter latency that appears occasionally indicates involvement of polysynaptic chains with a relatively low safety factor of propagation

The pattern of excitatory convergence illustrated in Fig 1 was typical of many cells in the border zone Other cells were discharged from the spinal fascicles only, or from one of the spinal fascicles and from one or several forelimb nerves Neurones were also found that received excitation only from one source, either the SCLT, the DC or one of the forelimb nerves, although focal potentials and activation of cells close by were obtained following stimulation of the SCLT, DC and forelimb nerves, which indicated that these cells were located in the border zone and not in the somatotopically organized part of the nucleus

When the synaptic action of a stimulus to the SCLT was studied intracellularly, it was repeatedly observed that the EPSP appeared with a constant size or not at all at stimulation intensities close to the threshold Fig 2 illustrates unitary EPSPs from a cell excited from the SCLT only With stimulus strength at the threshold value of the thalamic response (A) it gave either no response, an EPSP of a constant amplitude or a somewhat larger EPSP that fired the neurone At a slightly higher stimulus intensity the same two EPSP levels were seen (B) The all or nothing character of the EPSPs indicates that these are unitary i e due to the excitatory synaptic action of the terminals of one single afferent fibre The records in Fig 2 show that in this cell the summation of only two unitary EPSPs was required in order to discharge a propagated action potential Therefore excitation of only two neurones in the SCLT seems necessary for a propagated discharge Since there was not visible break in the summated EPSP the fibres exciting the cell apparently had nearly the same conduction velocity The fibres in the SCLT seem to constitute a rather homogeneous group as is also evident from the small rise in the stimulus intensity necessary to obtain a maximal response as compared with the threshold response

(Landgren *et al* 1965) The constancy of the latency and amplitude of the excitatory action from the SCLT is further illustrated in Fig 2, C Here the cell was also anti dromically discharged from the cortex (S II) A following stimulus to SCLT gives a unitary EPSP or no response at all

Although unitary EPSPs were most commonly obtained in response to stimula tion of the SCLT, such EPSPs were also observed in response to stimulation of the *forelimb nerves or the DC One cell, excited from the DC only, seemed to be fired* by one unitary EPSP When the cell was studied intracellularly, the action potential was found to be fired on the crest of the EPSP, or there was no response at all It should, however be noted that stimulation of the DC or the forelimb nerves in most cells gave an EPSP that grew continuously as the stimulus strength was increased above the threshold value until it reached the firing level

In summary, our findings indicate that there are cells in the border zone which can give a propagated discharge by the excitatory action of one or two afferent fibres most often belonging to the SCLT

Cortical connections

In order to investigate the cortical connections of the cells in the border zone brief electrical shocks were applied to the somatosensory areas S I and S II as well as to the surrounding cortex by means of a set of bipolar electrodes the poles of which were equally spaced The effects on the thalamic cells were analyzed with regard to anssynaptic and antidromic activation The criteria used for this classification latency of discharge the configuration of the spike and the ability to follow high frequency stimulation

It was often observed that a cell in the border zone could be excited from two distinctly different cortical points Such a cell is illustrated in Fig 3 This cell was excited from SCLT (latency 5 9 msec) and from DC (latency 6 6 msec) The cell was tested for excitatory effects from the cortical points shown by the drawing in Fig 3, and with the current intensities in mA as indicated for each point The cell could be discharged only from the three points indicated with filled circles The records obtained in response to stimulation of these points are shown in Fig 3, A—C The cell was fired with the lowest stimulus intensity (1 2 mA) from point A in the medial part of S I The latency was 3 2 msec (Fig 3 A) When the stimulu *was applied to point B laterally and caudally to point A the cell discharged ir* regularly and only if the stimulus intensity was increased to 16 5 mA i e 14 times but the latency was not significantly changed (Fig 3 B) The cell was also discharged from an area in S II (cortical point C) separated from the focus in S I by an inex citable zone The stimulus strength required in S II was 5 mA and the latency of the spike was 1 4 msec The antidromic nature of the response is suggested by the configuration of the spike which rises from the baseline without any visible prepoten tial There is, however a high break in the rising phase of the spikes due to change in risetime between the initial segment (IS) and the soma dendritic (SD) spikes A soma-dendritic blockage is seen in Fig 3 D

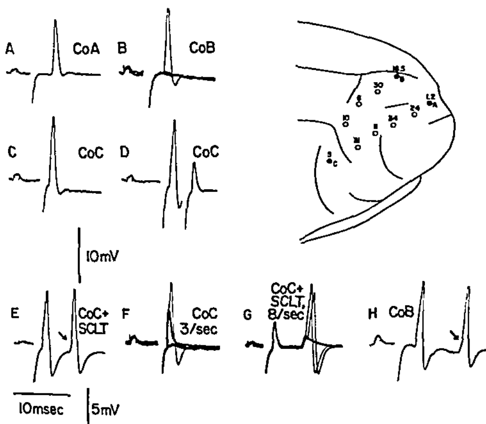


Fig 3 Intracellular records from a cell antidromically invaded from the cortical areas S I and S II The diagram to the right shows the location of the stimulated points and the stimulus strength in mV No short latency responses were obtained from points indicated with open circles Records A B and C were obtained from the points marked A B and C (filled circles) with thresholds as indicated Record D shows SD blockage to the second stimulus when paired shocks (interval 4 msec) were applied to point C. Record E shows simultaneous stimulation of the SCLT and the cortical point C

The difference in the configuration between antidromic and transsynaptic activation of this cell is illustrated in Fig 3 E—H In E the SCLT and the cortical point C were stimulated simultaneously with a stimulation rate of 1 per second Again, the first spike, elicited by the cortical stimulation arose from the baseline without any prepotential and there was a pronounced break between the IS and SD spikes of the rising phase The second spike evoked by stimulation of the SCLT, was postsynaptic It arose from a prepotential, indicated with an arrow and had a very small break on its rising phase In Fig 3 F are records showing stimulation at

old intensity of the cortical point C at a repetition rate of 3 per second In one out of 10 trials a full spike was elicited, in the other the spike showed either a SD blockage or there was no spike discharges at all and no signs of an EPSP In Fig 3 G the cortical point C was stimulated at higher intensity together with the SCLT and at a repetition rate of 8 per second In every sweep the cortical stimulation gave an IS spike The SCLT response consisted of an EPSP upon which the action potential occasionally appeared When the cortical point B was stimulated with high intensity, the cell sometimes discharged two action potentials (Fig 3 H) The first spike arose from the baseline without any prepotential but with a distinct break in the rising phase The later spike had a prepotential indicated with an arrow, of similar amplitude as the EPSP elicited from the SCLT but no clear IS—SD break Although the latency of the spike response elicited from the points A and B in S I was long the records in Fig 3 strongly indicate that this cell was fired antidromically from these points as well as from point C in S II

The cell was also tested for its ability to follow high frequencies given as double pulses with different intervals This particular cell followed paired stimulation of point C with an interval of 4 msec between the pulses but the SD component of the response to the second stimulus was then blocked (Fig 3 D) An ability to follow cortical stimulation at frequencies as high as 600—800 per second was observed in some antidromically activated cells Others were however blocked at frequencies above 10 per second An inability to follow high frequency stimulation is to be expected because of the efficient and longlasting inhibitory action induced in thalamic cells by cortical stimulation (Andersen *et al* 1964 a) Since some cells followed transsynaptic activation at frequencies of several hundred stimuli per second frequency following is not a good test for the differentiation between antidromic and transsynaptic activations

Throughout the experiments 27 out of 39 systematically tested cells were invaded antidromically from the somatosensory areas 14 of these cells were invaded from both S I and S II 8 cells only from S I and 5 cells only from S II Thus the results of our experiments have shown that a large number of cells in the spino-cervico-lemniscal border zone are discharged from two distinct foci in the hindlimb areas of S I and S II at a low stimulus intensity while other cells are antidromically invaded from only one of these areas When the cells were discharged from both S I and S II the foci were separated by a cortical region stimulation of which caused the thalamic cells to respond only occasionally when very high stimulus intensities were used The threshold for an antidromic activation of the SCLT cells was generally 2—3 mA although in several cells values below 1 mA were effective The shortest latency was usually found when S II was stimulated In many cells the threshold for the transsynaptic activation was lower than that of the antidromic response

Inhibition

The somatosensory relay cells in the main part of the ventro-basal complex of the thalamus are subject to a powerful recurrent inhibition Andersen *et al* 1964 a)

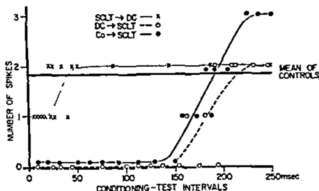


Fig 4 Plotted curves showing the degree and time course of inhibition of the test response when preceded by a conditioning stimulus to SCLT, DC or the hindlimb area of S I The cell was excited by SCLT and DC but not from cortex

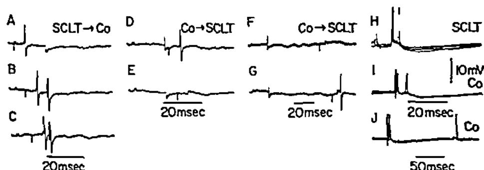


Fig 5 Extra (A—G) and intracellular (H—J) records of a cell showing excitatory as well as inhibitory actions from SCLT and the hindlimb area of S I In records A—C a stimulus to SCLT precedes the cortical stimulation in records D—G the conditioning stimulus is given to cortex Record H shows the intracellular response to a SCLT volley records I and J the response to stimulation of S I Membrane potential 50 mV

The cells in the border zone seem to constitute a less homogeneous group with regard to inhibition Many cells with excitatory convergence or activated from only one peripheral source showed the same type of recurrent inhibition as the cells in the main part of the nucleus in being inhibited for 100—200 msec after stimulation of the afferent pathways as well as of the somatosensory cortex In other cells, no signs of inhibition were found, either from the cortex or from peripheral sources The most common pattern of inhibition was, however, that the inhibition elicited by stimulation of the SCLT was much weaker than that produced by the DC, the forelimb nerves or the cortex The plotted curves in Fig 4 illustrate the inhibition observed in a cell which was excited by the SCLT and the DC pathway The inhibition is given as a reduction in the number of spikes discharged by the test volley When the SCLT volley preceded the stimulus to the DC the inhibition was weak and lasted less than 50 msec A conditioning stimulus to DC however inhibited the response from SCLT completely for 150 msec Although stimulation of the cortex did not discharge this cell, a conditioning stimulus to the hindlimb area of S I also inhibited the response from SCLT for about 150 msec The recovery from the in-

hibition after a conditioning stimulus to the cortex was followed by a period of hyperexcitability with increased test discharges This hyperexcitability appeared in many cells as a late discharge to the conditioning stimulus and with a latency of 100—200 msec (Fig 5 J) (cf Andersen *et al* 1964 a)

Sometimes a more complicated interaction between the cortical effect and the effect of a peripheral stimulus was observed Fig 5 illustrates a cell activated by the SCLT and cortex The cell was first studied extracellularly (records A—G) and later intracellularly (records H—J) During the extracellular recordings the cell was invaded antidromically from the hindlimb area of S I only when preceded by a stimulus to the SCLT at certain intervals (Fig 5 A—C) The facilitation of the antidromic response was due to an afterdepolarization following the discharge of the action potential by SCLT and preceding the hyperpolarization (H) When the cortical stimulation precedes the stimulus to SCLT a similar phenomenon was observed and there was a delay in the onset of inhibition of the SCLT response (D) As seen in the intracellular records (I and J) the cortical stimulus gave first an antidromic spike followed by a later transsynaptic action which occasionally discharged a second action potential Thus, there is evidence of a short lasting mutual facilitation from the periphery and the cortex on relay cells in the border zone This facilitation was followed by a hyperpolarization which was short lasting and weak when induced by the SCLT (H) The hyperpolarization elicited from cortex was more prominent and inhibited the SCLT response for 50 msec (Fig 5 D—G) The duration of the inhibition of the SCLT response was much shorter than the duration of the hyperpolarization (J) and the SCLT response is depressed only during the period of maximum hyperpolarization This is an indication of the strong synaptic linkage in the excitatory path which can break through a moderate degree of inhibition Fig 5 J also shows that the cortical stimulus induces a post inhibitory rebound excitation of a border zone cell

Discussion

The present investigation is concerned with properties of cells in a part of VPL lateral to the region which receives a somatotopically organized projection from the dorsal columns The cells are characterized by a considerable convergence with regard to their excitation by stimulation of afferent pathways Thus, cells are activated from the contralateral spino-cervico-lemniscal and dorsal column pathways as well as from contralateral forelimb nerves either by one of these or by two or several in different combinations Landgren *et al* 1965) In the present experiments only cells excited by stimulation of SCLT alone or in combination with other peripheral inputs have been investigated

Many thalamic cells excited by the SCLT were also excited from restricted foci in the cortical areas S I and S II at low stimulus intensities The short latency action potential arose from the baseline without any prepotential in an all or nothing fashion and often showed a clear IS—SD break These are characteristics of antidromic invasion of the cells Furthermore, the response elicited by cortical stimula

tion was compared with the response evoked transsynaptically by peripheral stimulation. The latter gave an action potential with a prepotential or only an EPSP at threshold intensities of the stimulus. Such a comparison supports the assumption of an antidromic invasion of cells in the lateral VPL following cortical stimulation.

The cortical response evoked by stimulation of the contralateral dorsolateral funiculus is mediated by the pathway of Morin (1955, *cf.* Andersson 1962). The first neurone of this pathway relays in the dorsal horn of the spinal cord of the same side from which it enters the cord (Lundberg 1964), the second neurone ascends in the lateral funiculus and relays in the lateral cervical nucleus. The third order neurone crosses the midline at C_1—C_3 and ascends in the medial lemniscus to the VPL of the thalamus (Morin and Thomas 1955, Busch 1961). The present results show that the thalamic relay of this pathway is located in the border zone in the lateral part of VPL. Although some fibres ascending in the dorsal column medial lemniscus also terminate in this region, the bulk of these fibres terminate somewhat more medially in VPL. So far, cells activated from the hindlimb component of the SCLT and antidromically invaded from cortex have been found only in this border zone of VPL.

According to the criteria given above, a number of cells in the lateral part of VPL were excited antidromically by stimulation at low intensities of restricted foci in both S I and S II. This can be explained on the assumption that the thalamic cells project upon the cortex with a divided axon, one branch reaching S I and the other S II. Since the distance from VPL to S I and S II is approximately the same, the difference in latency between the invasion from the two cortical areas suggests that the branches have different conduction velocities and thus different diameters. Stimulation of S II usually gave an invasion of the cell with shorter latency than did stimulation of S I. Therefore it seems possible that some thalamic cells project to S II with their main axon while an axon collateral projects to S I. Evidence for divided axons was also found by Andersson, Landgren and Wolsk (1966) in their study of the cortical connections of the thalamic cells relaying group I input from the forelimb and in *nucleus centralis posteromedialis* Darian Smith (1964) found some cells which could be antidromically invaded from the face areas of both S I and S II.

Rose and Woolsey (1958) studied the degenerations in thalamus after cortical ablations. They frequently observed small focal degenerations in the posterior part of the ventro basal complex when S II was removed. If in addition the auditory cortex was ablated, the degenerative changes in this part of thalamus were most severe, although ablation of the auditory cortex itself only gave minor changes. The possibility exists that this type of facultative degeneration (named sustained degeneration by Rose and Woolsey) is due to a cortical projection with divided axons.

The cortical response evoked by impulses in the SCLT arising from the lower part of the spinal cord has a latency about 3 msec shorter than the response elicited by impulses in the DC pathway (Mark and Steiner 1958, Norrsell [illegible]

1962, Andersson 1962) This latency difference may partially be due to a difference in the synaptic transmission The efficiency of a synapse does not only depend on the magnitude of the depolarization produced by the incoming volley, but also on the rate at which depolarization occurs (Sasaki and Otani 1961) The recordings from the cells activated by the SCLT showed that this pathway gave an EPSP with a much steeper rising phase than the EPSP elicited by the DC pathway Thus, the SCLT usually creates an EPSP that produces less accommodation of the membrane than does the DC pathway The postsynaptic depolarization obtained from SCLT often occurred in unitary steps which most likely are due to the release of transmitter substance from a single SCLT axon Summation of two or three unitary EPSPs elicited an action potential arising from the crest of the EPSP Similarly large unitary EPSPs have also been observed in the lateral geniculate nucleus (Bishop, Burke and Davies 1962) No obvious time dispersion was found between the individual EPSP units This is probably due to similar conduction velocities in the different SCLT fibres (Landgren *et al* 1965) The amplitude of the EPSP obtained from the DC or the forelimb nerves usually grew gradually with increasing stimulus strength, and its rate of rise was slower than the SCLT-induced EPSP In these circumstances accommodation of the membrane becomes an important factor, the safety factor of the synaptic transmission decreases with a concomitant increased synaptic delay

The longlasting postexcitatory inhibition with repetitive afterdischarges of thalamic cells described by Andersen *et al* (1964 a) was also found in the lateral border zone of VPL However, the inhibitory action elicited from the SCLT was usually weaker than that obtained from the DC pathway or from the forelimb nerves This difference was particularly obvious in cells with excitatory convergence Cortical stimulation elicited a strong inhibitory action of short latency in most of the cells in the border zone suggesting that the inhibition is effected by recurrent hyperpolarization Since a large number of thalamic cells are invaded by an antidromic volley, the cortical stimulation is expected to give strong recurrent inhibition The difference between the inhibitory action obtained from different peripheral sources may be due to differences in their excitatory connections Thus, if the SCLT axons excite only a few thalamo-cortical neurones compared with those excited by the DC path way only a small number of recurrent collaterals will excite interneurones with inhibitory action on the thalamic relay cells Consequently the inhibition will be more restricted than would be the case if the afferent volley excited a large number of thalamo-cortical fibres (cf Andersen *et al* 1964 a) An alternative explanation may be sought in a different number of inhibitory interneurones in various parts of the VPL, and also in a varying degree of their axonal ramifications

This work was supported by the Swedish Medical Research Council Project 14 91 0? and by a travelling grant to one of us (P A) from Letterstedtska Foreningen

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Acta physiol. scand. 1966. 68. 84 — 95
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Studies on the Mechanism for the Calorigenic Effect of Adrenaline in Man

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Received 7 February 1966

Abstract

SVEDMYR, N *Studies on the mechanism for the calorigenic effect of adrenaline in man Acta physiol scand* 1966 *68* 84--95

the calorigenic effect such as the heart stimulating and hyperglycemic effects of adrenaline is also considered

The calorigenic effect of catecholamines (CA) in animals and man have long been studied (reviews Lundholm 1949, Griffith 1951 Ellis 1956, Lundholm and Mohme-Lundholm 1960, Lundholm, Mohme-Lundholm and Svedmyr 1966) These studies indicate that the calorigenic effect of CA is probably not ascribable to one specific mechanism but constitutes the sum of several different effects, the relative importance of which may vary with the experimental conditions, animal species and CA derivate

The way in which adrenaline (A stimulates the oxygen consumption in man is of interest Notable among the mechanisms which *a priori* may be of importance for this action are the glycogenolytic and lipolytic effects of A

In experiments on the rabbit, Lundholm (1949) found that increased lactic acid production and metabolism could to a large part explain the calorigenic effect of adrenaline in this species In later experiments in man Bearn, Billing and Sherlock (1951) found that the blood lactate concentration and the oxygen consumption increased during adrenaline infusion The oxygen consumption of the liver was doubled, while at the same time its extraction of lactic acid increased Noradrenaline had a weaker effect on the liver oxygen consumption

Cobbold and Ginsburg (1960) found, however, that both noradrenaline (NA) and isoprenaline stimulated the oxygen consumption in man without increasing the blood lactate concentration appreciably They therefore doubted the significance of increased lactic acid metabolism for the CA calorigenic effect

Steinberg (1963), Steinberg *et al* (1964) demonstrated a parallelism between the calorigenic effect of NA and its capacity to increase the plasma free fatty acid (FFA) concentration, and also showed that NA increased the oxidation of C^{14} labelled palmitic acid Pronethalol blocked both the calorigenic and the FFA-mobilizing effect They suggested that the calorigenic effect of NA was induced by increased metabolism of FFA

Nicotinic acid (nic ac) has been shown to inhibit the FFA-mobilizing effect of CA but not its circulatory actions (Carlson and Oro 1962) Havel *et al* (1964) selectively blocked the FFA-increasing effect of NA with nicotinic acid and found that the calorigenic effect of NA was thereby approximately halved

The experiments described indicate that both increased lactic acid metabolism and FFA metabolism may be of importance for the calorigenic effect of CA in man

In connection with studies on the influence of thyroid hormones on the calorigenic effect of adrenaline in rabbit and man (Svedmyr 1966 a, b, c, d) it seemed of interest to determine the relative importance of the mechanisms discussed above for the calorigenic effect of A in man

In the present study, therefore, attempt was made to determine how great a role an increase in lactic acid metabolism and in FFA metabolism, respectively, played in the calorigenic effect of A in man The contribution of the lactic acid metabolism was calculated by means of a regression equation which correlated the increase in oxygen consumption and increase in blood lactate concentration These values were obtained on infusion of L(+)lactate in a quantity which gave about the same increase in blood lactate concentration as during A infusion The FFA increase could not be reproduced by FFA infusion since free fatty acids are too toxic to administer i v The FFA mobilizing effect of A was therefore selectively blocked with nicotinic acid and the consequent reduction in the calorigenic effect of A was determined

Method

The experiments were performed on a total of 6 healthy subjects of ages 22—30 years Studies were made of ...

... thermostatically controlled at 24 0 ± 0 5 C A plastic catheter was inserted into a cubital vein in each arm One of the catheters was used for the withdrawal of blood samples via a threeway tap and infusion of nic ac and the other for infusion of A or lactate When the catheters were not being used for these purposes 0 9 % NaCl solution without addition of heparin was infused into each of them After insertion of the catheters and commencement of NaCl infusion the subject rested for 60 min after which the basal metabolism was measured during two ... periods during each ... an values of these basal samples were taken as ... then changed in the first series ... A which was ... solved in 0 9 NaCl solution ... which 0 1 % ... The metabolism was subsequently determined ... 85 9 and 11 ... min after the start the A infusion Blood samples 7 ml for analysis of blood lactate blood glucose and plasma F

Fig 1 Influence of A (●–●–●–●) nicotinic acid (△ – – △) and nicotinic acid combined with A (○– – –○) on O_2 consumption CO_2 elimination and ventilation A in a dose of 0 10 µg/kg/min between 0 and 30 min Nicotinic acid in a dose of 5 mg/min between –30 and 120 min Every point is the mean of 5 expts

were taken 10, 20 30 45 60 90 and 120 min after the start of the A infusion In the second and third series nic ac was infused i v in a dose of 5 mg/min The infusion was commenced after the two basal samples had been taken and continued for 150 min when the experiment ended Ten min after the start of the nic ac infusion a blood sample was taken and between about 15 and 25 min after the start the metabolism was measured after which a further blood sample was taken After 30 min of the nic ac infusion (time = 0 in figures and tables) the experiment was identical with series 1 above apart from the fact that the nic ac infusion continued to the end of the experiment and that in series 2 only the 0 9 % NaCl infusion was given In series 3 on the other hand from time O A infusion was started and nic ac infusion was continued The mean difference in the A effect with and without nic ac was calculated in the same subjects Prior to the nic ac experiments the subjects were given 0 5 g nic ac orally three times daily for three days so that they would become accustomed to the flushing which occurred during the nic ac infusion

[illegible] basal samples [illegible] This last series [illegible] 10 min intervals

The O_2 consumption, CO_2 elimination and ventilation were measured with a Hartman and Braun's metabolic recorder This instrument records continuously the difference between the O_2 and CO_2 contents in the inspired and expired air from variations in heat conduction capacity The ventilation was measured with a low friction gasometer All these parameters were recorded with a Grass [illegible] ventilation [illegible] and 760 mm

The blood lactate concentration [illegible] Lundholm and Vamos (1963), the blood glucose concentration according to Bergmeyer and Bernt 1962 and the plasma FFA concentration according to Trout Estes and Friedberg 1961

Results

Effect of 0 10 µg A/kg min infused for 30 min The effect of this A infusion on different physiological variables is shown in Fig 1 and 2 and also Table I and II On infusion of A the oxygen consumption rose considerably as quickly as within 5 min and then

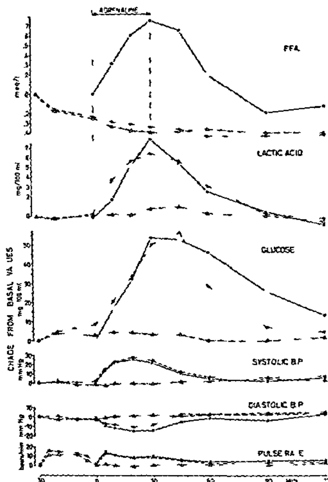


Fig. 2 Influence of A (●—● ●—●) nicotinic acid (△ △) and nicotinic acid combined with A (○---○) on some metabolic and circulatory functions in man. For further informations see Fig. 1

continued to increase reaching its maximum at the end of the infusion. It then decreased and regained the basal value 60 min after the end of the infusion. The CO_2 production did not run parallel with the O_2 consumption but reached its maximum as quickly as after 5 min decreasing then relatively more rapidly than the O_2 consumption. The ventilation increase ran parallel with the increase in CO_2 production. The pulse frequency increased 10—15 beats min, the systolic blood pressure rose and the diastolic blood pressure fell during the infusion (Fig. 2).

The plasma FFA concentration rose continuously during the infusion, the maximal increase being 750 μeq l. The blood lactate concentration rose almost linearly with the infusion time and showed a maximal increase of 8 mg at the end of the infusion; 60 min later it regained the basal level. The blood glucose concentration showed a maximal increase of 54 mg at the end of the infusion and then decreased slowly but the basal level was still no

TABLE I The influence of adrenaline (Adr), nicotinic acid (Nic. ac.) and Nic. ac. and Adr in combination on O_2 consumption, CO_2 elimination and respiratory minute volume. Mean values ±S.E. in 5 fasted subjects. Adr 0.10 μg/kg/min infused for 30 min. Nic. ac. 5 mg/min infused for 150 min. Time in relation to the start of Adr infusion. P = probability that the effect was due to chance.

	Adr (I)	Adr+Nic. ac. (II)	Nic. ac. (III)	Mean difference in Adr effects with and without Nic. ac. I-(II-III)
O_2 ml/kg/min				
Basal values	3.55±0.0823	3.47±0.084	3.41±0.128	
Change from basal values after −10–0 min		0.01±0.020	0.00±0.066	
0–10	0.48±0.039 P<0.001	0.35±0.045 P<0.005	−0.02±0.040	0.11±0.075
25–35	0.75±0.072 P<0.001	0.47±0.065 P<0.005	0.00±0.041	0.29±0.126
43–48	0.48±0.084 P<0.005	0.30±0.051 P<0.005	−0.06±0.080	0.11±0.072
55–65	0.25±0.114	0.14±0.066	−0.03±0.030	0.09±0.10[illegible]
85–95	0.00±0.098	0.04±0.052	−0.02±0.036	−0.06±0.121
CO_2 ml/kg/min				
Basal values	2.76±0.083	2.73±0.087	2.72±0.151	
Change from basal values after −10–0 min		0.02±0.044	−0.03±0.069	
0–10	0.83±0.039 P<0.001	0.91±0.123 P<0.005	0.00±0.014	−0.08±0.078
25–35	0.63±0.061 P<0.001	0.78±0.086 P<0.001	0.11±0.053	−0.04±0.121
43–48	0.09±0.049	0.09±0.037	0.04±0.025	0.05±0.059
55–65	0.06±0.049	0.11±0.085	0.05±0.041	−0.01±0.081
85–95	0.05±0.036	0.16±0.051 P<0.05	0.21±0.050 P<0.02	0.10±0.072
Respiration volume l/min				
Basal values	5.77±0.167	5.55±0.197	5.64±0.244	
Change from basal values after *−10–0 min*		*0.57±0.118* P<0.01	*0.11±0.120*	
0–10	1.80±0.111 P<0.001	2.24±0.309 P<0.005	0.24±0.140	−0.20±0.268
25–35	1.46±0.185 P<0.005	2.13±0.279 P<0.005	0.51±0.107 P<0.01	0.16±0.108
43–48	0.28±0.124	0.86±0.099 P<0.001	0.44±0.082 P<0.01	−0.13±0.15[illegible]
55–65	0.29±0.124	0.71±0.25 P<0.05	0.38±0.166	−0.04±0.159
85–95	0.38±0.120 P<0.05	0.82±0.127 P<0.005	0.60±0.114 P<0.01	0.16±0.136

TABLE II The influence of adrenaline (ADR), nicotinic acid (Nic ac) and Nic ac and ADR in combination on the FFA glucose and lactic acid content of the blood. Symbols as in table I

	ADR (I)	ADR + Nic ac (II)	Nic ac (III)	Mean difference in ADR effects with and without Nic. a I (II III)
FFA μeq /l				
Basal values	686±75.6	638±106.3	583±62.0	
Mean change from basal values after −5 min		−226±68.1	−258±38.3	
		P < 0.05	P < 0.005	
+10	317±120.6	−278±98.9	−334±41.5	262±187.7
		P < 0.05	P < 0.005	
30	768±162.9	−336−95.9	396±47.9	708±195.4
	P < 0.01	P < 0.05	P < 0.005	P < 0.05
45	661±187.5	−360±97.6	379±42.0	642±183.8
	P < 0.05	P < 0.05	P < 0.001	P < 0.05
60	202±128.6	−434±111.8	−367±45.1	274±141.8
		P < 0.02	P < 0.005	
90	−192±93.6	−430±119.1	−406±53.6	−168±118.8
		P < 0.05	P < 0.005	
Glucose mg/100 ml				
Basal values	78±4.8	81±3.5	81±2.5	
Mean change from basal values after −5 min		8±6.0	3±1.3	
+10	17±2.7	21+10.6	5±1.5	1±10.9
	P < 0.005	P 0.05	P < 0.05	
30	54±6.9	50−7.5	4±1.9	7±7.2
	P < 0.005	P < 0.005		
45	53±4.3	57±12.9	3±1.7	−1±9.6
	P < 0.001	P 0.02		
60	46±2.9	29±7.7	0±2.8	17±8.3
	P < 0.001	P < 0.02		
90	26±4.5	7−8.7	0±2.1	19±9.7
	P 0.005			
Lactic acid mg 100 ml				
Basal values	6.8−1.17	5.4±0.76	4.5 0.31	
Mean change from basal values after −5 min		0.2±0.65	0.3±0.48	
−10	1.7±0.51	3.6_1.50	0.2 0.37	−1.7−1.63
	P < 0.05			
30	8.0±1.04	4−0.94	0 0.49	2.2−0.99
	P < 0.005	P 0.015		
45	5.5±0.58	5.6±0.98	0.9 0.61	0 _0.85
	P < 0.001	P 0.005		
60	2.5−0.53	3.1±0.57	2 0.41	−0.4−0.68
	P < 0.01	P 0.01		
90	0.4±0.44	0.14±0.98	0.1 0.35	−0.4±0.73

Effect of nicotinic acid infusion Infusion of nic ac in a dose of 5 mg/min for 150 min provoked an initial flush which gradually disappeared No definite change in the oxygen consumption was noted (Fig 1, Table I) The CO_2 elimination, on the other hand, began to increase about 30 min after commencement of the nic ac infusion The respiratory quotient thereby increased from a mean basal value of 0 76 to a maximum of 0 82 The difference 0 06 ± 0 019 was significant ($P < 0 05$) The ventilation and CO_2 production increase ran parallel

Nic ac reduced the plasma FFA concentration considerably, and after about 60 min this concentration reached a new level which lay approximately 350 μeq/l below the initial value The blood glucose concentration increased very slightly, while the blood lactate concentration showed no significant change (Fig 2, Table II) The pulse frequency increased initially by 10—15 beats/min but there was no alteration in blood pressure (Fig 2)

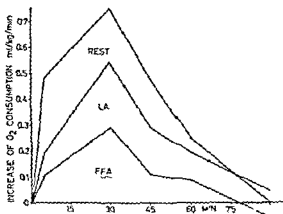
Infusion of nicotinic acid + adrenaline When the subject was given nic ac before and during the A infusion the adrenaline effect on the oxygen consumption was reduced (Fig 1, Table I), and this reduction was most pronounced at the end of the A infusion period, when in the subjects treated with A alone the plasma FFA concentration reached its maximum In the nic ac treated subjects the elevating effect of A on FFA was completely inhibited (Fig 2, Table II)

The mean difference in A effect (0—45 min) on the O_2consumption in tests on the same person without and with nic ac was 0 15 ± 0 038 ml/kg/min ($P < 0 02$, $n = 5$)

In Table I and II the sum of the separate effects of nic ac and A are also compared with the effect of these drugs in combination A reduction of the O_2 consumption (0 15 ± 0 048 ml O_2 kg min $P < 0 05$) was shown when the summed mean values during the period 0—60 min were compared On the other hand nic ac caused no reduction of the A induced increase in CO_2 production The R Q were higher in the nic ac + A experiments than in the A experiments (mean values 0 94 and 0 87 after 10 min respectively) During A infusion, however the R Q must be evaluated with some caution part of the increased CO_2 production is probably due to the fact that CO_2 is driven out from the bicarbonate of the blood and tissues owing to the increase in different acids lactic acid and FFA) induced by the A infusion This relationship has been studied in more detail by Lundholm and Svedmyr (1966 a) In the nic ac — A experiments the plasma FFA increase was inhibited, however It seems probably, therefore that the R Q increase in these experiments as compared with the A experiments was a true one In that case this should indicate an increase of the carbohydrate metabolism in the nic ac — A experiments Some support for this assumption is also provided by the course of the blood glucose concentration, which decreased considerably more rapidly in the nic ac A experiments after discontinuation of the A infusion than in the A experiments Fig 2, Table II) The effect of A infusion on the blood lactate concentration was not influenced by the nic ac treatment

The respiratory volume in the nic ac — A experiments showed approximately

Fig 3 Tentative presentation of the relative importance of FFA and lactic acid (L A) metabolism for the calorigenic effect of A in man. FFA Part of the calorigenic effect of A which was blocked by nicotinic acid and attributed to increased FFA metabolism L A Increase of oxygen consumption calculated from the relationship between the increase of lactic acid content in the blood and the stimulation of oxygen consumption as found by Svedmyr (1966 b) The hyperglycemic effect of A and its stimulation of the heart work may contribute to the Rest effect



the same course as the CO_2 production, which substantiates previous findings (Lundholm and Svedmyr 1966 a) of a relationship between the respiration-stimulating effect of A and its influence on CO_2 production The effects of the adrenaline on pulse and systolic blood pressure were not influenced by nic ac , but the reduction of the diastolic pressure was smaller after nic ac (Fig 2)

Effect of L(+)lactate on oxygen consumption and blood lactate concentration in man Preliminary experiments indicated that on infusion of 2 3 mg L(+)lactate/kg/min the blood lactate concentration increased to approximately the same degree as with adrenaline infusion It is of interest that this lactate quantity agrees with the maximal lactic acid elimination from the forearm tissues which was induced by an adrenaline infusion in the same dose (Lundholm and Svedmyr 1966 b) On infusion of this quantity of L(+)lactate the blood lactate concentration increased maximally by 9 4 mg % (8 0 after A) and the oxygen concentration by 10 % (21 % after A) These experiments are reported in more detail in another paper (Svedmyr 1966 c) When the increase in blood lactate concentration was correlated with the increase in oxygen consumption in these experiments the following regression equation was obtained $Y = 0.028X + 0.032$ Y = increase of O_2 consumption in ml kg min over the basal level and X = increase of blood lactate concentration (in mg % over the basal value) On calculating by means of this equation the increase in oxygen consumption that could be expected to be induced on the basis of increased blood lactate concentration on infusion of A, the curve in Fig 3 was obtained The area below this curve in the period 0—90 min after the start of the infusion was 35 %, of that below the corresponding A curve

Discussion

FFA metabolism After treatment with nic ac the calorigenic effect of A in man was reduced and at the same time its FFA mobilizing effect was selectively inhibited The results agree with the findings of Havel *et al* (1964) with regard to the calorigenic

effect of NA in man In Fig 3 the proportion of the calorigenic effect of A which was blocked by nic ac has been marked out by FFA This proportion constitutes 23 % of the total effect in these tests An important question is whether the blockade of the FFA metabolism had caused the relative inhibition of the calorigenic effect This seems probable since nic ac did not inhibit any other effects of A, but there is no conclusive evidence Had it been feasible by infusion of free fatty acids to raise the plasma level to the same degree as after the A infusion it might have been possible to study this question further, but free fatty acids are too toxic to be administered in this way (Oro and Wretlind 1961), and if they had been given together with albumin the fluid volume infused would have been very large

Assuming that there is a causative relationship between FFA mobilization and calorigenic effect, the stimulation of the oxidative processes might perhaps be interpreted as a result of increased access to substrate Steinberg (1963) found in experiments in vitro that the oxidation of FFA increased with an increase of the FFA concentration in the medium This was not accompanied by any increase in oxygen consumption, however and this effect was therefore probably associated with reduced utilization of other substrates It is well known that there is an inverse relationship between, e g, the FFA and glucose utilization of cardiac muscle (Opie, Evans and Shipp 1961 Randle 1963 In my experiments, for example, nic ac reduced the plasma FFA concentration considerably under basal conditions without influencing the basal O_2 consumption The R Q rose, however indicating that the oxidation of carbohydrate and/or protein had increased Challoner and Steinberg (1966) have however recently found that an increased FFA concentration in the perfusate increased the O_2 consumption of isolated rat heart

It has also been demonstrated that NA considerably increases the turnover rate of plasma FFA Havel 1964, Steinberg et al 1964 The increased efflux of FFA from plasma is also combined with an increase in the incorporation of FFA to glycerides Adrenaline raises the blood glucose concentration, and it is well known that increase in this concentration increases the resynthesis of triglycerides (Steinberg 1963 CA should therefore stimulate an FFA cycle with an increase in both hydrolysis and resynthesis of triglycerides This cycle is energy-consuming and it has been suggested by Ball and Jungas 1961, Havel 1964 and Mayoro 1966) to be partly responsible for the calorigenic effect of CA It has been reported that NA, in in vivo experiments increases the triglyceride concentration in the liver, heart and muscle Carlson Liljedahl and Wirsen 1965

Further studies of this problem are however required in order to determine more quantitatively the relative importance of direct FFA oxidation and/or the resynthesis of FFA to triglycerides for the calorigenic effect of CA

Lactate metabolism

An important question is whether a lactate infusion can completely emulate the effect exerted by A on the lactate metabolism This question is closely connected with that of whether lactate production and lactate oxidation take place in different

tissues and how an increase in lactate metabolism induces stimulation of the oxygen consumption According to the 'lactate cycle" theory of Cori (1931) A induces glycogenolysis in skeletal muscle after which the lactic acid produced is transported via the blood to the liver where it is to the greater part resynthesized to glycogen The stimulation of oxygen consumption by the lactate would thus be due to energy losses on resynthesis of the lactate to glycogen Adrenaline would thus stimulate both a "lactate cycle' and a 'FFA cycle" The doubling of the oxygen consumption of the liver and the increase of its uptake of lactate which has been demonstrated in man by Bearn, Billing and Sherlock (1951) during an A infusion could be explained, at least in part, by an increased lactate metabolism NA also stimulated the oxygen consumption of the liver, — the effect was about one third as great as that of A — and therefore an increased FFA metabolism may have contributed to these effects

If Cori s 'lactate cycle" theory is correct the effect of a lactate infusion should emulate fairly well that of A, on the lactate metabolism More recent studies appear to indicate, however, that the lactate metabolism is more complicated than is outlined above

In experiments on the rabbit Drury and Wick (1965) found that the major part (65 %) of infused C^{14} labelled L(+)lactate is oxidized to CO_2 and that only a few per cent were found in the liver and muscle glycogen In eviscerated animals the 'turnover rate' of radioactively labelled lactate was about 8 times slower than in animal with an intact liver The animals were still able to oxidize lactate, however, though with greatly increased lactate concentrations in the blood Even if the liver is the most important organ for lactate oxidation, it can also be metabolized to some extent by other organs such as heart, kidney and brain (Brin 1965, Levy 1965, Sacks 1965) It has not been shown, however, that lactate stimulates the oxygen consumption in these organs However, in adipose tissue CA stimulates both the oxygen consumption, lactic acid production and FFA release, and lactate also stimulates the oxygen consumption of adipose tissue (Lynn McLeod and Brown 1960 Havel 1964) It is therefore possible that both lactate production and lactate oxidation may take place in combination with stimulation of the O_2 consumption in adipose tissue If this is so then the lactate concentration in the tissues should not be completely reflected by the blood lactate concentration Lactate diffuses relatively slowly and considerably concentration difference in lactate concentration between blood and tissues have been shown (Sacks 1937, Lundholm 1956) It is therefore possible that the lactate concentration in adipose and possibly other tissues was higher on infusion of A than of lactate Such a possibility might have given rise to some undervaluation of the role of lactate in the calorigenic effect of adrenaline

Schimassek (1965) has found that A and glucagon stimulated lactate uptake and O_2 consumption of the liver to a considerable extent Exton and Park 1965) reported that phosphoenolpyruvate carboxykinase had a rate limiting effect on the transformation of lactate to glucose by the rat liver The activity of this enzyme was stimulated by glucagon, A and cyclic 3 —5 AMP It is therefore probable that A a stimulating effect on the lactate uptake and resynthesis by the liver S

effect could partly explain the differences in the results of Cori (1931) and Drury and Wick (1965) with regard to lactate metabolism

Other mechanisms As may be seen in Fig 3 the total calorigenic effect of A was somewhat greater than the sum of the calorigenic effects that could be assumed to be due to stimulation of the FFA and lactate metabolism It is possible that the role of the lactate metabolism was undervaluated and that the magnitude of this "residual effect" is not so great as is indicated in Fig 3 It is probable, however, that other mechanisms than those discussed above may have been of contributory importance

Adrenalin increased the heart frequency by about 10 beats/min (Fig 2) and has also been found to increase the cardiac output by 50—100 % (Goldenberg *et al* 1948) The respiratory volume also increased It is highly probable that these increased muscular effects may be associated with stimulation of the oxygen consumption, but with the data available at present it is difficult to exactly calculate their part in the calorigenic effect The hyperglycemic effect of A is probably of some importance for its calorigenic action, too (Boothby and Sandiford 1923) and this will be evaluated in a further study Adrenaline also increased the oxygen consumption of the forearm musculature in man (Lundholm and Svedmyr 1966 b) and it has not as yet been established whether this effect was due to an increase in the lactate or FFA metabolism or induced by a more direct stimulation of the muscle metabolism

The work was supported by the Swedish Medical Research Council and the Faculty of Medicine University of Goteborg I am grateful for the technical assistance of Miss Inga Johansson and Mr Theodor Dzedin

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Electrophysiological and Micro-electrophoretic Studies on Sympathetic Preganglionic Neurones in the Spinal Cord

By

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Received 14 February 1966

Abstract

Hongo, T and R W Ryall *Electrophysiological and micro-electrophoretic studies on sympathetic preganglionic neurones in the spinal cord* Acta physiol scand 1966 *68* 96—104

Preganglionic sympathetic neurones in the thoracic cord of the spinal cat were located by recording antidromic extracellular potentials in response to stimulation of preganglionic fibres in the superior splanchnic nerve Antidromic potentials were only recorded in the region of the intermediolateral cell column Few units were firing spontaneously or could be orthodromically activated The conduction velocities in the preganglionic axons of units from which recordings were obtained ranged from 1 5 to 9 m/sec Local micro-electrophoretic applications of DL-homocysteic acid fired the cells but noradrenaline had no excitatory effect and did not inhibit spontaneous synaptic or antidromic firing or the firing to the excitant amino acid Acetylcholine had no effect on the few cells tested

Noradrenaline NA is a potent inhibitory substance when applied micro-electrophoretically to some interneurones in the lumbar region of the spinal cord of the cat (Engberg and Ryall 1965, 1966 and there was some correspondence between the distribution of sensitive interneurones and the relative densities of NA containing terminals in this region of the cord This and other evidence led to the suggestion that NA is an inhibitory transmitter liberated from the terminals of descending fibres (Engberg and Ryall 1965 1966) There is a high density of NA containing terminals in the intermediolateral column of the spinal cord Dahlstrom and Fuxe 1965) and the preganglionic sympathetic fibres are known to arise from cell bodies located in this region (see Mitchell 1953 It was therefore of interest to determine the sensitivity of the preganglionic sympathetic neurones to NA When this investigation was carried out there was no published account of the electrophysiological properties of these neurones However Fernandez de Molina Kuno and Perl (1965) have

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[2] Supported by grants from IBRO UNESCO and the Wellcome Trust

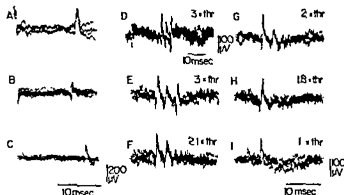


Fig 1 Types of antidromic potentials recorded in the thoracic cord to antidromic stimulation of fibres in the superior splanchnic nerve A unitary negative potential B positive negative potential probably unitary C unitary negative positive potential (stimulus straddling threshold) D—I compound potentials produced from several units at different intensities of stimulation ranging from 1 to 3 times threshold for the first short latency potential (I) Note expanded sweep in E—I In A—D the stimulus artifact occurs near the beginning of each sweep

recently reported on the properties of neurones antidromicall activated from the white rami Many of the present results with antidromic stimulation of the splanchnic nerve confirm their findings Thus the present results will be presented in comparison with those of Fernandez de Molina *et al* (1965), with special attention being paid to those observations which are complementary to those of the latter authors a preliminar account has been published (Hongo and Ryall 1966)

Methods

Cats were operated upon under ether anesthesia The spinal cord was exposed from the upper border of the fifth thoracic segment down to the lower part of the ninth thoracic segment and the dorsal roots were cut ipsilaterally to the recording side Recordings were usually made at the seventh thoracic segment and occasionally the central stump of the dorsal root of the same segment was mo n ed f

… euromuscular blockade was maintained by i v injections of gallamine triethiodide A pneumothorax was routinely employed The majority of the experiments were carried out with 5 barrelled micro-electrodes which had overall tip diameters of 4—6 µ The recording and electrophoretic techniques were the same as those used in a previous investigation (Engberg and Ryall 1966) However some of the recordings were made with single barrelled electrodes (tip diameter approximately 1 µ) filled with 3M sodium chloride Square wave pulses of 0 3—0 5 msec duration were used for stimulation of the preganglionic fibres Recordings of volleys in the peripheral stumps of the dorsal and ventral roots were usually made at the end of the experiment and the length of the splanchnic nerve was measured in order to determine the conduction velocities

All extracellularly recorded potentials are shown with negativity upwards

Results

Antidromically generated potentials

It was sometimes difficult to locate extracellular potentials which could be attributed to potentials generated in sympathetic neurones by antidromic volleys in the ipsilateral splanchnic nerve This was probably due to the small size of the cells and their dendrites (Fernandez de M lina *et al* 1965 and therefore of the poten

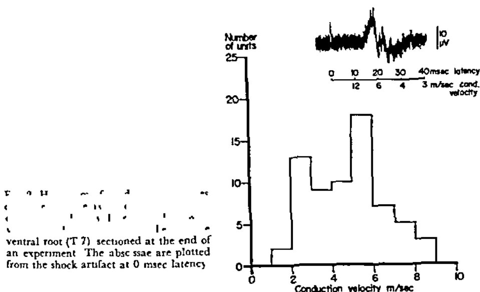
and to the variation in the size of the cell groups at different levels of the cord and in different cats

Potentials were only considered to be antidromically generated if they satisfied the following criteria, 1) the latency from the stimulus artifact was invariable at a constant frequency of stimulation and was compatible with the speed of antidromic propagation of volleys in the preganglionic nerve 2) units followed a reasonably high frequency of stimulation (at least 100/sec) compatible with unimpaired conduction of the antidromic volleys

Focal potentials recorded from groups of cells were small and probably generated mainly by only a few cells These potentials were always irregular in outline (Fig 1 D) and were composed of several negative waves These waves could be differentiated by adjusting the stimulus intensity, the units with the longest latency firing with the largest stimulus and *vice versa* An example of such a focal potential is shown in Fig 1 D—I In I, the stimulus was just threshold for a unit responding with the shortest latency (17 msec) in the group The first wave is probably due mainly to this unit since the size of the potential did not increase markedly when the stimulus intensity was increased At 1 8 times threshold a second potential (latency 19 5 msec) became evident This second potential increased stepwise with increase in stimulus intensity up to 2 1 times threshold for the first unit At 2 0 times threshold a third small potential (latency 22 msec) may be seen, but this did not increase when the stimulus was increased and at 3 0 times threshold the third potential is partially masked by a fourth (latency 23 2 msec) which like the potential with the shortest latency appeared to be generated mainly by a single unit Also of interest in these records is the small positivity preceding each negative potential but these were not always present

The unitary spikes were often recorded in the absence of potentials from other cells (Fig 1 A—C) They were usually predominantly negative and were far smaller 50—200 μV exceptionally 300 μV) than those reported by Fernandez de Molina *et al* 1965 The recording conditions were relatively stable the same unitary spikes could be observed for more than 30 min An initial positivity was frequently observed as seen in Fig 1 B D—I which however appeared to have a gradual onset and was usually much smaller than the following negativity Occasionally unitary spikes with a late positivity were also encountered (Fig 1 C) The rise time and the duration of the unitary spikes as measured from the start of the negativity was 0 8 ± 0 2 standard deviation 61 determinations) msec and 2 3 ± 0 6 (58 determinations msec respectively The small late positivity (not always present) was too small in relation to the noise level to allow measurement

Many other neurones were n[illegible] t dr[illegible]n cally act vated from the ipsilateral splanchn[illegible] erve but could be orthodromicall[illegible] ed [illegible] st mulat on of the contralateral splanchn c nerve dorsal [illegible] a latency [illegible] th the [illegible] ulus [illegible] in af [illegible] at n [illegible] msec)



ventral root (T 7) sectioned at the end of an experiment The absc ssae are plotted from the shock artifact at 0 msec latency

and variable latencies were encountered occasionally This was especially the case when the ipsi lateral splanchnic nerve was stimulated in preparat ons n which the cord was not transected at both T5 and T9 These d scharges of presumably synaptic orig n probably represent an afferent inflo from lower segments of the cord but the possibil ty of activation via axon collaterals of the

Latency of antidromic potentials

The antidromic axonal conduction velocities for sixty seven units are plotted as a frequency histogram in Fig 2 Also shown in Fig 2 is a record of the small volley recorded in the peripheral stump of the ventral root when the splanchnic nerve was stimulated at the end of an experiment

The range of velocities was from 1 5 to 9 m/sec (Fig 2 which was similar to the distribution of conduction velocities of the antidromic volleys recorded in the ven tral root (Fig 2, inset record) The range is similar to that reported by Fernandez de Molina *et al* (1965) who stimulated the white rami Faster conducting volleys equivalent to the P1 subgroup (11—15 m sec as defined by these authors were not clearly found in the compound ventral root potentials in the present study

Spontaneous firing and synaptic activation of preganglionic neurones

The majority of antidromically activated units did not fire spontaneously and could not be fired by stimulation of either the central end of the ipsilateral dorsal root or by stimulation of the contralateral splanchnic nerve with single shocks (contralateral dorsal roots intact However in contrast with the experiments of Fernandez de Molina *et al* (1965 a few were found which could be fired synapti cally or were spontaneously active with a low frequency of firing (less than 10 sec) Such spike discharges could be



Fig 3 Extracellular spike potentials from a preganglionic neurone showing the collision between evoked orthodromic potentials and an incoming antidromic volley In all records the splanchnic nerve was stimulated near the beginning of each sweep (indicated as artifact) The neurone fired antidromically at 26 5 msec latency (A B) and was sometimes fired orthodromically by a dorsal root volley (C D) *In B, the dorsal root volley* did not elicit a spike and the antidromic potential was seen with the same latency as in A In C and D orthodromic potentials were generated and no antidromic potentials were observed The axonal conduction velocity of this neurone was 3 8 m/sec

vated unit by the similar sizes and shapes of the action potentials evoked spontaneously, orthodromically and antidromically, and, more conclusively, by the collision between the orthodromically and antidromically evoked spikes This collision is illustrated in Fig 3 for one neurone, which was activated with a variable latency by dorsal root stimulation In records A–D the ipsilateral splanchnic nerve was stimulated antidromically near the beginning of each sweep An antidromic potential with a latency of 26 5 msec (conduction velocity 3 8 m/sec) (A) was only observed when dorsal root stimulation failed to elicit a spike (B) Similarly, a collision between orthodromically propagating but spontaneously generated potentials and antidromic potentials was demonstrated on spontaneously firing units

The location of the antidromic potentials

The records in Fig 4 were obtained in one of two cats in which the area over which antidromic potentials could be recorded was relatively large compared with other experiments, although it was still small in absolute terms (approximately 300 μ in diameter) The area in which single unitary spikes were recorded was even smaller However, antidromic potentials could sometimes be recorded over a distance of 200 μ In every experiment the location of the electrode in the cord was determined by fixation and sectioning of the cord with the electrode tip *in situ* Invariably, the antidromic potentials were only recorded from a small area in the intermediolateral region of the grey matter, as shown in Fig 4 In this diagram the depth from the cord dorsum is only approximate since the degree of shrinkage which occurred when the cord was fixed and embedded was not known but was assumed

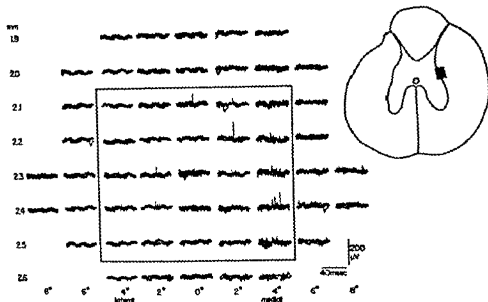


Fig 4 Extracellular antidromic focal potentials recorded at different depths from the cord dorsum in tracks with the insertion spot kept constant but at different relative angles The antidromic potentials were only observed within the area indicated by the square The area of this square is indicated by the shaded area in the diagram to the right of the figure The depth of the microelectrode tip from the cord dorsum is indicated in mm and the relative tracking angles of the micro-electrode are shown below the figure

to be about fifteen per cent Inspection of stained histological sections showed that the size of the intermedio-lateral horn varied markedly at different levels along the axis of the cord and even at the same level on opposite sides of the spinal cord This variation probably explains the difficulty in locating the preganglionic neurones, and the low cell density may to some extent explain the small focal potentials Furthermore most of the cells in the intermedio-lateral column were very small (less than 20 μ in diameter)

The action of noradrenaline on sympathetic preganglionic neurones

NA was administered micro-electrophoretically with currents up to 80 × 10[illegible]A through a 1 M solution of the bitartrate salt while recording antidromic unitary potentials from twelve neurones In no instance was there any effect on the antidromic firing This shows that NA is not a particularly potent inhibitory substance on these cells Furthermore excitation was never observed Antidromic firing may not be a very sensitive test for a slight inhibitory action because of a high safety factor for antidromic invasion Spontaneous firing should be a more sensitive index but was rarely observed However NA was tested on three occasions [illegible] cells which were firing spontaneously and had spikes sufficiently large 100—300 μV to allow discrimination from the background noise Again no effect was observed with noradrenaline On two cells [illegible] activation of the preganglionic

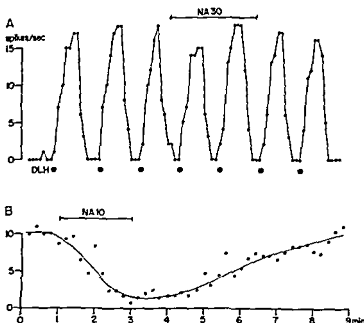


Fig 5 A effect of NA on the firing to DLH of a sympathetic preganglionic neurone DLH was ejected from the micro-electrode for 30 sec at each dot NA (30 × 10 ⁹A) was applied during the period indicated by the horizontal bar B effect of NA on the synaptic firing of another unit to stimulation of contralateral splanchnic nerve (contralateral dorsal root intact) This unit was not a preganglionic neurone The contralateral nerve was stimulated at a frequency of 4 sec and each stimulus produced 2 or 3 spikes The numbers of spikes in each second (averaged over 12 sec) are plotted as ordinates in the figure

neurone from the dorsal root was also unaffected Quiescent preganglionic neurones could be fired by the micro-electrophoretic application of an excitant amino acid, DL-homocysteic acid Fig 5 A shows the effect produced by repeated brief ejections of this substance on the firing of a neurone which was invaded antidromically from the splanchnic nerve During the period indicated by the horizontal bar, NA was also ejected with a current of 30 10 ⁹A but it neither excited the cell nor did it reduce the firing to the amino acid However, it was only possible to carry out such an examination on three cells since it was usually difficult to maintain the size of the extracellular potentials constant for a sufficiently long period This inability to maintain the spike size constant was partly due to the fact that small movements of the spinal cord in relation to the micro electrode had a marked effect on the spike amplitude such movements were often troublesome, *despite* the routine use of a pneumothorax, particularly towards the end of a long experiment when pulsating movements of the cord became more prominent Another difficulty was that the excitant amino acid often caused a rapid decrease in spike size in the concentrations required to fire the cells

In contrast, many other types of neurone may be inhibited by NA applied micro-electrophoretically Engberg and Ryall, 1965, 1966) The neurone in Fig 5 B was activated by stimulation of the contralateral splanchnic nerve at a frequency

of 4/sec, but was not invaded antidromically from the ipsilateral nerve and lay dorsal to the intermedio lateral region Each stimulus produced a discharge of one to three spikes and the total number of spikes in one second (averaged over 12 sec) are plotted as ordinates in the figure During the application of NA with a current of only 10 × 10 $^{-9}$A the synaptic discharge was virtually abolished, and when the application ceased there was a slow recovery

Acetylcholine was tested by micro-electrophoretic administration on four preganglionic neurones, but was inactive

Discussion

The electrophysiological studies reported in this paper confirm and extend those of Fernandez de Molina *et al* (1965) In the present investigation the extracellularly recorded potentials were usually small and predominantly negative They were usually stable enough to allow observations for a considerable time In contrast, Fernandez de Molina *et al* (1965) obtained large (up to 15 mV) and initially positive extracellular potentials, or »giant extracellular potentials» (Granit and Phillips 1956, Freygang 1958, Freygang and Frank 1959) The duration of the unitary spikes obtained in the present study (2 3 ± 0 6 msec) was considerably shorter than that reported by these authors This is not unexpected since the recording conditions are clearly different it is very likely that the tip of the micro-electrode was positioned in close proximity to and probably invaginating the cell membrane in their study However, it should be noted that the duration of the extracellular negative potential of the sympathetic preganglionic neurones is still long The duration of the equivalent extracellular potential in other neurones is about 1 msec in spinal motoneurones (Fatt 1957 Nelson and Frank 1964 and 0 48 ± 0 16 (standard deviation, 18 determinations msec in neurones of the spinocervical tract (Hongo and Lundberg unpublished

Since antidromic potentials were only recorded from the intermedio-lateral region of the grey matter there was no evidence to support the suggestion that there may be another group of preganglionic neurones located more medially see Mitchell 1953) There was a variation in the size of the area from which antidromic potentials were recorded along the axis of the cord but no attempt was made to correlate this observation with variations in the size of the intermedio-lateral cell column, which were seen histologically

Fernandez de Molina *et al* 1965 found no unit which could be orthodromically activated and in the present investigation only a few preganglionic neurones could be activated orthodromically by stimulation of dorsal root fibres These extracellularly recorded orthodromic potentials were positively identified as belonging to the preganglionic neurones by colliding orthodromic and antidromic volleys The conduction velocity of axons in these units did not belong to the P_1 subgroup (11—15 m sec as was suggested by *Fernandez de Molina et al* 1965 Since so few units were orthodromically activated it seems likely that only a small proportion of the preganglionic neurones can participate in segmental autonomic

reflexes in this preparation. It is possible that in the spinal preparation the segmental reflex pathways to the preganglionic neurones are partially inhibited.

The failure of NA to affect antidromic firing of the preganglionic neurones is evidence that it was not a powerful inhibitory substance. There was, in addition, no evidence for an excitatory action. Unfortunately, spontaneous or synaptic firing, which should be more sensitive tests for inhibition, was rarely observed in these preparations, but was unaffected by NA on the few occasions when tests were possible. Similarly, firing by an excitant amino acid was also unaffected by NA. With the techniques used in this investigation it was possible to show that NA has marked inhibitory actions on many lumbar interneurones (Ryall 1965, Engberg and Ryall 1965, 1966) and on interneurones in the thoracic cord which were fired by afferents in the splanchnic nerves. Thus the failure to affect the firing of the preganglionic neurones with NA is unlikely to be due to technical difficulties in administration.

These studies have shown that NA has no action on at least some of the cells giving rise to preganglionic fibres in the splanchnic nerve. Thus the results suggest that the NA terminals in the intermedio lateral column of the thoracic cord (Dahlstrom and Fuxe 1965) are ending on neurones which are not those giving rise to the preganglionic fibres in the splanchnic nerve. The possibility still remains that the noradrenergic innervation occurs on preganglionic neurones synapsing in the sympathetic trunk ganglia.

It is a pleasure to acknowledge the helpful advice and criticism given by Prof. A. Lundberg in the preparation of the manuscript.
This work was supported by the Swedish Medical Research Council (Project No 14X 91 02A).

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Acta physiol. scand 1966. 68. 105—117

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Glucose Uptake in Potassium-Depolarized Mammalian Muscle

By

CHRISTIAN CRONE

Received 19 February 1966

Abstract

CRONE, C *Glucose uptake in potassium-depolarized mammalian muscle* Acta physiol scand 1966 *68* 105—117

Experiments were performed [illegible]

The striated muscle cell displays a clear selectivity to the entry of non-electrolytes Some molecules enter the cell interior faster than others of comparable size and physical properties — probably due to interaction with components in the cell membrane Glucose is an example of a hydrophilic substance which enters faster than do other hexoses The nature of the interaction between cell membrane and permeant molecule is completely unknown and therefore, it has so far been almost impossible to elucidate the mechanism by which insulin enhances the rate of passage of glucose into striated muscle

In order to throw some light on the nature of the facilitated passage of glucose experiments were performed in which the effect on the muscular glucose uptake of increasing the extracellular potassium concentration was studied The idea behind the work was originally that the depolarization as such might increase the permeability of the membrane to glucose, but the work later focussed on the possible connection between the glucose uptake and the processes in the cell membrane responsible for sodium extrusion and potassium uptake

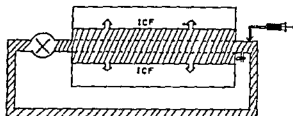


Fig 1 Diagram of experimental set up Blood diluted with cat Ringer is circulated by a pump Material passes from the extracellular space into the intracellular space (ICF) Glucose is continually added by means of a motordriven syringe

The general plan for the experiments was to determine both the uptake of glucose and the oxygen consumption in an isolated perfused cat hind limb before and after addition of potassium and in other experiments to see whether interference with ion transport by addition of strophanthin affected the glucose uptake

Methods

Experimental technique and calculations 61 expts were performed on cats starved for 18 hrs Usually

a bubble oxygenator with a gas mixture (saturated with water vapour) containing 5 6 CO_2 and oxygen The blood was circulated with a Sigma motor pump The composition of the Ringer solution added to the blood in the control period was (meq/l) Na 150 K 4 1 Mg 2 6 Ca 1 8 Cl 130 HCO_3 24 H_2PO_4 1 5 SO_4 2 6 When potassium rich solution was used sodium chloride was substituted with potassium chloride

In the earlier experiments Rheomacrodex[R1] was added to provide the necessary colloid osmotic pressure but as it was found that this substance greatly increased the inulin blank value it was given up and the potassium rich perfusate did not thereafter contain high molecular material Generally one third of the original perfusate was substituted with potassium rich perfusate so that the protein concentration in the mixed blood only fell by 30 % The initial potassium concentration after the substitution was about 35 -45 mM

Fig 1 shows schematically the experimental set up Glucose was infused continually in amounts corresponding to the uptake so that the concentration in the extracellular fluid remained almost constant during the control period The uptake was calculated from the continually infused amount of glucose When the concentration fell or rose the product of the difference in glucose concentration times the extracellular space was added to or subtracted from this value

The oxygen consumption was calculated as the arterio-venous difference times the blood flow It was determined once in the control period and twice or three times in the potassium period The R Q was determined as the ratio between the CO_2 content and O_2 content in the blood sample drawn in the control period The extracellular space was determined with inulin after 1 1/2 hrs equilibration

[illegible]

1400 ml The strophanthin doses were between 5 10 mg except in a few experiments in which either doses of 25 mg or of 3 mg were tried with no obvious difference in effect

The membrane potential of single muscle fibres was measured in some of the experiments by means of a micropipette filled with 3 M KCl Pipettes with a tip potential above 5 mV were not used The pipettes were filled according to the destillation procedure described by Caldwell and [illegible] and the signal [illegible] he pipettes were [illegible] n and the deep [illegible] was inserted

1 H Hovgård, Pharmacia Ltd kindly supplied the Rheomacrodex

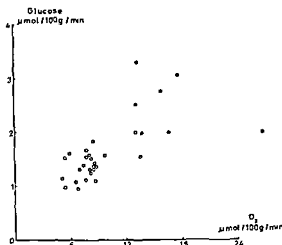


Fig 2 Glucose uptake and oxygen consumption Open circles Control periods Filled circles Experiments with increased plasma potassium concentration. Every point represents average values from one experiment

holme Model K1) *Inulin* was determined by means of Bojesen's colorimetric procedure (1954) *Lactic acid* was analyzed enzymatically as described by Lundholm Mohme-Lundholm and Vamos (1963)

Results

1 *Experiments with potassium depolarization*

a *Control period* The hind limb was perfused for 1—1 1 2 hrs before potassium-rich blood was substituted The results from 24 expts in which both the glucose uptake and the oxygen consumption was determined are shown in Fig 2 The glucose uptake is plotted as a function of the oxygen consumption

The average glucose uptake was 1 4 µmole/100 g min (S D 0 25, n = 24 The oxygen consumption was 8 1 µmoles/100 g/min (S D 1 6, n = 24) The R Q was 0 75 (S D 0 15, n= 24) The low R Q shows convincingly that substrates other than glucose were used

The glucose uptake in muscle depends among other factors on the plasma concentration of glucose as shown by Lundsgaard Nielsen and Orskov (1939 This point was reinvestigated and it was found that when the glucose concentration went up from about 100 mg/100 ml to 300 mg 100 ml the uptake rose from 1 4 to 1 7 µmoles/100 g/min As the glucose concentration often rose immediately after the substitution with potassium it is of importance to know that a rise in concentration of about 20—30 mg/100 ml has only a very limited effect on the uptake

The plasma concentration of potassium stayed almost constant in the control period, a small loss of potassium led to an increase of about 1 mM during one hours perfusion The lactic acid concentration was also constant during this period, being

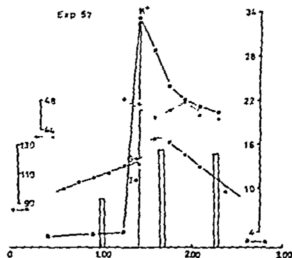


Fig 3 Variations in plasma concentration of glucose (●——●) potassium (○——○) and inulin (+——+) during a continuous infusion of 4 mg glucose per minute before and after potassium substitution The vertical line labelled K shows time of substitution with potassium rich blood I and G inulin and glucose concentration in potassium rich blood used for substitution Glucose mg/100 ml Inulin mg/100 ml Potassium meq/l The vertical bars show the oxygen comsumption The oxygen consumption in the control period was 6 6 μmol/100 g/min Abscissa hours

between 20 and 30 mg 100 ml If anything there was a slight tendency to a fall Fig 3 shows the concentration of glucose and potassium in an experiment which is representative

b *Potassium depolarization* When part of the perfusion fluid was substituted with potassium-rich blood the potassium concentration rose within 2—3 min to an initial level of 35—45 mM During the subsequent 1—2 hrs the potassium concentration fell and levelled at 20—25 mM Fig 3 shows the time course of the potassium concentration after substitution

The substitution with potassium-rich blood was accompanied by an increase in glucose uptake The uptake rose from an average of 1 5 μmoles/100 g min to 3 0 (S D 1 00, n = 20, an average increase of 100 %, The steady fall in glucose concentration in the potassium-period compared with the slightly rising concentration in the control period is clearly seen on Fig 3 which also shows an initial sharp rise in glucose concentration which often followed substitution with potassium rich blood This early period lasted for 10—15 min and was succeded by a period of 1 1 2 hrs with increased glucose uptake There was no apparent connection between the increased glucose uptake and the potassium concentration at the rather high potassium concentrations which were established in these experiments The early rise in glucose concentration was undoubtedly due to a rapid shrinkage of the extracellular space as revealed by a simultaneous rise in inulin concentration inulin was added to the substituting potassium rich blood so that the concentration was close to that at the end of the control period A rise in inulin concentration after the substitution, therefore, signified shrinkage of the extracellular space The shrinkage was more or less complete after 20—30 min During this period the muscle is gaining KCl until the internal [K] [Cl] product equals the external value The typical variation in inulin concentration is shown on Fig 4 The average rise in inulin concentration after the substitution was 18 8 in 10 expts in which there was a good correspond-

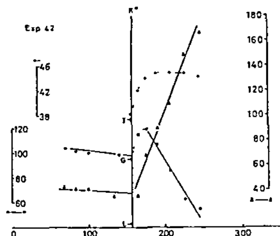


Fig 4 Variations in plasma concentration of glucose (●———●) inulin (+ — — — +) and lactic acid (▲———▲) before and after potassium substitution The vertical line labelled K shows time of potassium substitution I G and L Inulin, glucose and lactic acid concentration in potassium rich blood used for substitution Inulin mg/100 ml Glucose mg/100 ml Lactic acid mg/100 ml Glucose was continually infused (4 4 mg/min) Abscissa hours

ence between the inulin concentration in the two portions of blood which were mixed As the relative rise in glucose concentration in the early period was of the same magnitude as the rise in inulin concentration it is most probable that there was no reduction in glucose uptake in the very early phase In many experiments the glucose concentration already began to fall during the early period of shrinkage of the extracellular space

The increased glucose uptake occurred together with an increased oxygen consumption which was 16 5 μmoles/100 g/min (S D 4 4, n = 8) The results are shown in Fig 2 The increase was thus of the same order (about 100 %) as the increase in glucose uptake although the values scatter much more In one experiment (which is not included in the calculation of the average) the oxygen consumption rose to 39 8 μmoles/100 g/min — more than 200 % of the resting consumption It was regularly observed that the oxygen consumption fell in the first 10 minutes after elevation of the potassium concentration The reduction was about 35 %

The increased oxygen consumption lasted for the whole period of observation (1—2 hrs) which makes it unlikely that the rise was caused by contracture due to the potassium-depolarization The muscle tension was not measured so that it is not possible to decide definitely whether there was actually a slight contracture It is however, improbable that the increased oxygen consumption was due to contracture Pillat, Kraupp, Giebisch and Stormann 1958) who perfused isolated cat hind limbs did not find any changes in the mechanical tension of the gastrocnemius muscle when the potassium concentration was increased to 20 meq l A reason for a lack of contracture in potassium depolarization under these circumstances was possibly that the potassium concentration outside the fibres only rose slowly as reflected in the time-course of the depolarization Hill and Howarth 1957 observed twitching in the frog sartorius when the potassium concentration was suddenly increased to 18 meq l The twitching came to an end within 2 min When the potassium concentration was altered more gradually no detectable twitching occurred The rat

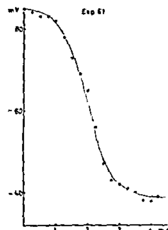


Fig 5 Time course of change of membrane potential in a muscle cell when part of the perfusate was substituted with potassium rich blood at 0-time The microelectrode was kept in position during the whole period of measurement

of depolarization was measured in several of the present experiments and it took about 2 minutes to build up a new potassium concentration outside the cells Fig 5 shows the temporal course of the rate of change of membrane potential in one experiment The fact that the intracellular micropipette remained in place during the period of potassium substitution speaks also against development of contracture in the muscle fibres

Fluid shifts accompanying potassium substitution As already mentioned shrinkage of the extracellular space took place immediately after potassium substitution As judged from the rise in inulin concentration (Fig 4) the extracellular space was reduced by 20 % The extracellular space averaged 300—350 ml so that a volume of 60—70 ml moved into the cells The intracellular space was not measured, but Huycke and Kruhoffer 1955) who used a similar preparation found a total water space of 900—1400, the intracellular volume would thus be of the order of 600—1000 ml The degree of cellular swelling could therefore not be above 10 % In order to see whether this swelling was responsible for the increased uptake of glucose, experiments were performed in which SO_4^{2-} was the dominant anion, but the effect on glucose uptake and on oxygen consumption was unchanged

2 *The effect of strophanthin poisoning*

The experiments reported so far only prove that potassium depolarization in mammalian muscle leads to an increase in glucose uptake which runs more or less in parallel with an increase in oxygen consumption The rise in glucose uptake could be secondary to the increase in oxidative metabolism Another possibility is that the transfer of glucose to the intracellular phase could somehow be coupled to the sodium extrusion mechanism it is known that potassium depolarization leads to an increased efflux of sodium ions Horowicz and Gerber 1965 a and b A third possibility is that the extra influx of glucose could be a consequence of an increased membrane permeability in the depolarized state

To differentiate between these possibilities experiments were carried out in which

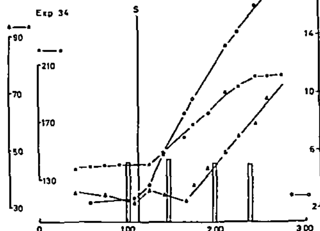


Fig 6 Experiment with strophanthin poisoning 8 mg strophanthin was added at S ●——● plasma glucose concentration (mg/100 ml) O——O plasma potassium concentration (mEq/l) △——△ concentration of lactic acid (mg/100 ml) The vertical bars show the oxygen consumption The oxygen consumption in the control period was 7 8 μmol/100 g/min Abscissa hours

the sodium extrusion mechanism was inhibited with strophanthin As usual the perfusion started with a control period lasting 1—1 1/2 hrs Then strophanthin was added to give a final concentration of 10^{-5} M in the total water phase After a time lag of 10—20 min a pronounced inhibition of the glucose uptake took place as reflected in a sharp rise in the extracellular concentration of glucose Fig 6 shows the results from an experiment of this type

The average uptake of glucose after strophanthin fell to 0 3 μmole/100 g min (S D 0 20, n = 13) The oxygen consumption was slightly depressed, being 6 9 μmoles 100 g/min (S D 2 65, n = 12) compared with an average uptake in the control period of 7 3 μmoles/100 g/min Fig 7 (open circles) shows the relation between glucose uptake and oxygen consumption in the strophanthin experiments It is seen that the glucose uptake was inhibited to a much larger extent than the oxygen consumption

The muscles began to lose potassium within 10 min after the addition of strophanthin (Fig 6) The average loss of potassium from the cells in 13 strophanthin expts was 4 2 μeq/100 g/min (S D 0 80, n = 13) as calculated from the rise in potassium concentration and the known value of the extracellular space The potassium loss occurred together with a gain of sodium of the same magnitude indicating that the oxygen uptake fell by 0 4 μmole under circumstances in which the sodium pump was blocked to the extent that about 4 2 μeq Na were gained per 100 g per min Thus in mammalian muscle 5 Na ions are pumped for each oxygen equivalent and only a small fraction of the oxygen uptake in mammalian muscle seems to be needed for processes concerned with sodium pumping

3 *Potassium depolarization after strophanthin*

When potassium depolarization was induced in the usual way *after* strophanthin administration the strong inhibition of the glucose uptake was eliminated The

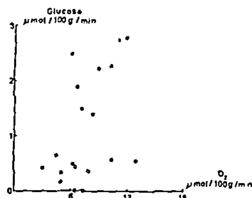


Fig 7 Glucose uptake and oxygen consumption Open circles after addition of strophanthin Filled circles experiments with potassium depolarization after a period with strophanthin

TABLE I Glucose uptake and oxygen consumption in perfused cat muscles under varying circumstances

	Glucose uptake µmoles/100 g/min	O_2-consumption µmoles/100 g/min
Control	1 4	8 1
	(S D 0 25 n 24)	(S D 1 60 n = 24)
K-depolarization	3 0	16 5
	(S D 1 00, n 20)	(S D 4 40 n 8)
Strophanthin 10^{-4} M	0 3	6 9
	(S D 0 20, n 13)	(S D 2 6, n 12)
K-depolarization after Stroph	2 1	8 5
	(S D 0 50 n 7)	(S D 2 06 n = 7)

glucose uptake went up to an average value of 2 1 µmoles/100 g/min (S D 0 50, n = 7) This is somewhat higher than the value in the control series (1 54 µmoles/100 g/min) The oxygen uptake under these circumstances was only moderately increased (Fig 7), the average being 8 5 µmoles 100 g min (S D 2 06, n = 7) Generally, potassium acts as if it overcomes the effect of strophanthin, but the usual high glucose uptake and oxygen consumption found after potassium depolarization in preparations which had not received strophanthin were not seen

Table I summarizes the results of the various types of experiments described in this and previous sections

Increased vascular resistance Hind limb perfusions can under normal circumstances continue for 4—5 hrs without any change in perfusion resistance When the potassium concentration was elevated the perfusion resistance always went up, as an immediate response After a few minutes the resistance began to fall again reaching the control level within 1/2—1 hr The perfusion pressure rose from the normal level of 80—100 mm Hg to about 200—250 mm Hg Addition of strophanthin also had this effect on the vascular resistance though the rise tended to be smaller Again the resistance

fell slowly, but in contrast to the potassium experiments it never returned to normal values When potassium depolarization was induced in strophanthin poisoned muscles a secondary rise in perfusion pressure took place The fall was now very slow and the vascular resistance always ended at a much higher level than in simple potassium or strophanthin experiments

The potassium dependent rise in perfusion pressure was preceded by a very small and rapid fall in pressure immediately after the addition of potassium (lasting for 1/2 min) This 'relaxation" was never seen preceding the 'strophanthin" rise

Lactic acid Both potassium depolarization and strophanthin administration led to an increase in lactic acid production Lactic acid production was a constant feature in the strophanthin experiments where the rate of lactic acid production (as judged from the accumulation in the extracellular fluid) was 1 89 μmoles/100 g/min (average of 10 expts) The lactic acid production started with a time lag of 20—30 min (Fig 6) which would imply that the lactic acid production was not a result of the arteriolar concentration (which was most pronounced immediately after the addition of strophanthin) The same argument applies to the lactic acid production observed during potassium-depolarization where the lactic acid production went on more or less unaffected by the fall in perfusion pressure which occurred after the initial rise In some of the experiments the arteriolar contraction only lasted for 10—15 min and yet the lactic acid production seemed to continue The lactic acid production in 9 potassium expts averaged 2 2 μmoles/100 g/min The production of lactic acid was not seen in all experiments and it never became possible completely to control this parameter

Discussion

The results reported in the previous section showed that it was possible to alter the rate with which glucose passes into muscle cells in various ways The main observations were that the glucose uptake rose parallel with a rise in oxygen consumption when the external potassium concentration was increased, and that strophanthin reduced the glucose uptake considerably without leading to a corresponding fall in oxygen consumption

Any interpretation of these results may place the cause either in the cell *interior* or in the cell *membrane* If the increased cellular metabolism was responsible for the observed variations in the glucose flux then the mechanism might be that increased glucose metabolism in the potassium experiments led to a fall in the concentration of glucose-6-phosphate This substance inhibits hexokinase and therefore phosphorylation would increase An increased intracellular phosphorylation could diminish the concentration of free glucose inside the cell and thus increase the transfer rate

The strophanthin experiments suggest another interpretation of the potassium effect on glucose uptake Strophanthin inhibits the active sodium transport as reflected in the steady loss of potassium from and increased net uptake of sodium in the cells The marked difference in the strophanthin effect on glucose uptake and oxidative metabolism makes it clear that glucose uptake is not necessarily a simple function

of the cellular metabolism and it is tempting to look at the inhibition of glucose uptake as being fundamentally related to the inhibition of the active transport of sodium (or active uptake of potassium) The rate of accumulation of potassium in the extracellular fluid averaged 4 2 μeq/100 g/min As the volume of fluid outside the cells did not change the potassium loss corresponds to the net gain of sodium If strophanthin leads to no change in the passive fluxes of sodium or potassium (and this is probable as the membrane potential does not change under the influence of strophanthin) the rate of sodium accumulation represents the inhibited part of the pumped sodium efflux In the same period the glucose uptake fell by 1 2 μmoles/100 g/min so if there is a stoichiometric relationship between ion transport and glucose uptake the results may suggest that 1 molecule of glucose is taken up whilst 3—4 Na ions are pumped out (or alternatively, 3—4 potassium ions are pumped inwards)

The possibility that the influx of a metabolically important non electrolyte is coupled to the ionic transport in the membrane is interesting because in this way the influx of glucose could automatically be geared to the needs of the cell Namely *when muscle is activated the pumped sodium efflux increases in the recovery phase* following an action potential (when the extra amount of sodium gained during the spike is pumped out) If the proposed mechanism is correct glucose influx would thus be coupled to the action potential which in itself is responsible for activation of the contractile energy requiring system in the cell interior This mechanism would adjust glucose influx to the cellular energy requirements under circumstances where the need for glucose suddenly rises considerably In this connection it is relevant to *mention that Holloszy and Narahara (1965) found an immediate rise in the uptake* of a carrier transported non metabolizable sugar 3 methylglucose in electrically stimulated frog sartorius

The idea that there is a connection between ion transport and glucose transport has received considerable attention since the report of Riklis and Quastel (1958) in which it was shown that the presence of Na ions is necessary for the intestinal absorption of glucose Crane (1962) proposed that glucose penetrates into the intestinal epithelial cell as a Na glucose complex Na is then pumped out in the lumen again and thus the sodium pump drives the glucose transport Schultz and Zalusky (1964) advanced a similar hypothesis Csaky (1963) showed that strophanthin inhibited glucose absorption in intestinal epithelium but he explains the Na sugar interaction in a different way regarding Na as being essential for that part of the transport system which converts chemical energy into pump activity Because it is necessary for the possible ATPase system sodium could be involved in up hill transport of glucose Sodium transport may be necessary for the transport of other substances than glucose thus Berndt and Beechwood (1965) found that the renal absorption of urate was strophanthin sensitive and Vogel Lauterbach and Kröger (1965) demonstrated the importance of sodium for the renal transport of glucose and of para amino-hippuric acid *In one respect the situation in muscle is different* from the above mentioned tissues as the glucose transport in this tissue is of an equilibrating type whilst in intestine and kidney it is of an up hill type

Clausen (1965) Sotringen stel lyde Clausen (1965) did not find any inhibition effect any inhibitory effect on isolated rat diaphragms with strophanthin in concentrations between 10^{-5}—10^{-6} M He found on the other hand a correlation between sodium concentration in the incubating medium and glucose uptake in isolated rat diaphragms His conclusion was, therefore that glucose entry may be a function of the passive influx of sodium This mechanism, however cannot explain the increased glucose uptake in potassium depolarized muscle as the Na influx is not altered under these circum tances (Horowicz and Gerber 1965a)

The interpretation given above is only one of several possible Another is that the *membrane potential* as such controls the glucose uptake as it does with sodium efflux (Horowicz and Gerber 1965 a and b) The results of the strophanthin experiments do not easily fit such a concept because the membrane potential does not change immediately as a result of the strophanthin administration Indirectly however the membrane potential may — by its control of sodium efflux — affect the glucose uptake

In the third type of experiments described in the previous ection potassium administration after strophanthin poisoning led to a significant increase in glucose uptake as if the strophanthin effect had thus been eliminated The glucose uptake increased to levels somewhat higher than the resting uptake with only a small increase in oxygen consumption (another situation in which these two parameters do not follow one another) Glynn (1957) found that in red cells potassium could overcome the strophanthin effect. Page Goerke and Storm (1964) demonstrated competition between strophanthin and potassium in cat heart muscle and the same appears to take place in muscle cells leading to the restoration of the ability to transport glucose During the strophanthin period the muscle cells gain sodium so that the activity of the sodium pumping system may be greater once the strophanthin effect is overcome by potassium — this may explain that the glu ose uptake was higher than in the control periods A more detailed analysis of this type of experiment is however not possible as long as the ion fluxes are unknown

Two other observations made during the experiments requir co mments

1) Strophanthin always caused arteriolar constriction soon after administration (despite the fact that the metabolic effect of strophanthin always required some time to develop) Mason (1963) found that strophanthin reduced forearm blood flow due to an increased vascular resistance and Waldhausen and Herendeen 1963 showed that the renal blood flow in dog [illegible] strophanthin because of an increased vascular resistance It has thus been proved in several cases that strophanthin can lead to contraction of smooth muscle presumably due to an increased spike activity (Casteels 1963

2) The observation that potassium depolarization enhances the production of lactic acid was originally made by Hegnauer Fenn and Cobb 1934 in isolated frog muscle and later confirmed by Hill and Howarth 195 and by Muller and Simon (1960) who also used *in vitro* system An increased pr duction of lactic acid was found in the majority of the exper m nt reported in this article It cannot be stated with

certainty whether this lactic acid production was due to the vasoconstriction which followed the potassium depolarization or whether is was due to other causes Among these a reduction of intracellular ATP concentration could be the essential mechanism (due to uncoupling of oxidative phosphorylation) Briner, Simon, Frater and Tasker (1959) found a reduced ATP content in muscle soaked in Ringer solutions with 20 mM potassium A lowered ATP concentration can lead to increased glycolysis because it is itself an inhibitor of phospho fructo-kinase

If the theory of a coupling between glucose transport and ion transport is correct then the old theory of phosphorylation as an intermediary step in glucose transport *gains a new significance* It has been argued *that phosphorylation - dephosphorylation* cannot be a necessary step in the transport because the hydroxyl-group specificity in active sugar transport is incompatible with the phosphorylation capacity of known kinases If, however, it is the sodium/potassium transport system which requires phosphorylation dephosphorylation (Skou 1964) then glucose transport is connected with phosphorylation in a way which does not imply phosphorylation of *the sugar molecule*

It is generally accepted that there are three known causes of increased glucose uptake in muscle cells insulin, muscular work and hypoxia To this list high external potassium concentration must now be added Future work will show whether the relation between ion transport and glucose transport will lead to a deeper understanding of the way in which glucose enters the musle cell Thus, the effect of insulin on glucose uptake might be secondary to an effect on pumped ion transport

Jens Astrup B A assisted in the measurements of muscle membrane potentials and his cooperation is thankfully acknowledged Financial support was received from the Danish Foundation for the Advancement of Science from F L Schmidt s Foundation and from Fonden til Lægevidenskabens Fremme

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Acta physiol. scand. 1966. 68. 118—126

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Motor Deficits after Transsection of a Bulbar Pyramid in the Cat

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Received 4 March 1966

Abstract

LAURSEN, A M and M WIESENDANGER *Motor deficits after transsection of a bulbar pyramid in the cat* Acta physiol scand 1966 *68* 118—126

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on the operated and unoperated side Placing reactions were delayed or absent in contralateral limbs struggling was diminished and there was a tendency for the paws to remain in awkward positions After one month operated cats could be distinguished from unoperated cats only by diminished flexor reflexes in contralateral legs and by a slight delay in contact placing Damage of the medial lemniscus produced the tendency to place contralateral paws in awkward positions lasting for one week Unlike pyramidal section lemniscal damage did not produce deficient flexor reflexes

There is agreement that pyramidotomy in cats produces motor dysfunction in the contralateral limbs which can be described roughly as abnormalities in gait and posture, in placing and hopping reactions, and as a tendency to extension of the limbs most clearly demonstrated when the animal is suspended in a sling with the legs hanging freely The signs are described fully and the literature reviewed by Marshall (1934), Tower 1935 and Liddell and Phillips (1944) There is disagreement as to the extent, duration and cause of the motor signs In Tower's animals the impairment was evident in such simple acts as struggling, clawing, scratching and walking and seemed to be permanent with no sign of further recovery after about one month Marshall's animals survived up to 4 weeks the degree of recovery in the various symptoms was different in each animal, but no constant set of symptoms was left at the end of the observation period

Liddell and Phillips animals survived up to one year and showed persistent increased resistance to pressure to the pad of the contralateral limbs, measured with a dynamometer, as well as inability to walk on a ladder or pipe, the affected limbs

TABLE I Motor deficits in extremities contralateral to pyramidotomy in 13 cats

Cat	Survival period in weeks	Flexor posture of pendant limbs	Struggling	Awkward positions of paws	Gait	Visual placing	Contact placing
A 1	4	Reduced	Reduced*	No	Normal	Delayed*	Delayed
A 2	8	Reduced	Reduced*	Yes*	Slipping*	Normal	Delayed
A 3	1	Reduced	Reduced*	No	Normal	Delayed*	Delayed
A 4	2	Reduced	Reduced*	Yes*	Slipping*	Delayed*	Absent
B 1	5	Reduced	Reduced*	Yes*	Dragging*	Delayed*	Delayed
B 2	10	Reduced	Reduced*	Yes*	Normal	Delayed*	Delayed
B 3	20	Reduced	Reduced*	Yes*	Slipping*	Delayed*	Delayed
B 4	7	Reduced	Reduced*	Yes*	Dragging*	Delayed*	Delayed
C 1	4	Reduced	Reduced*	No	Normal	Delayed*	Delayed
C 2	2	Reduced	Reduced*	No	Normal	Delayed*	Delayed
C 3	14	Reduced	Reduced*	No	Slipping*	Normal	Delayed
C 4	5	Normal	Normal	No	Normal	Normal	Normal
C 5	4	Reduced	Reduced*		Normal	Normal	Delayed

* Returning to normal after one week

slipping off and remaining helplessly protruded downwards Tower considered the fundamental loss of function to be a loss of phasic activity, chiefly flexor whereas Ranson (1932) and Liddell and Phillips (1944) ascribed their findings to extensor hypertonus

Tower attributed the greater severity and the permanence of the motor defect in her animals to the fact that the pyramidotomies in her series were complete whereas some of Marhall s were incomplete Involvement of the medial lemniscus was a feature of the lesion in both series and was described as massive in Tower s series Liddell and Phillips (1944) thought absence of hypertonia could be due to damage or oedema involving the inferior olive or the medial fillet

We have investigated cats with complete and incomplete section of one bulbar pyramid and cats with combined lesions of one pyramid and of the medial lemniscus in the medulla oblongata, animals with stereotactic lesions of the medial lemniscus in the mesencephalon served as controls Slow motion cinematography and recording of muscle action potentials were used to analyze movements and posture

Material and Methods

Surgical procedures Experiments were made on 16 cats 1 8 3 kg operated under pentobarbital sodium anesthesia In 13 cats the pyramid was exposed by a ventral approach Schiff 1859 Liddell and Phillips 1944 was viewed through an operating microscope and cut with a knife at the level of the trapezoid body Incision of the dura often produced bleeding from tiny dural vessels which was stopped by compressing the vessel with a fine forceps for 2 min No other bleeding was encountered All animals were treated with terramycin until they were afebrile for 3 7 days In 3 cats an electrolytic lesion (2 5 mA DC 20 sec was placed within the mesencephalic portion of the

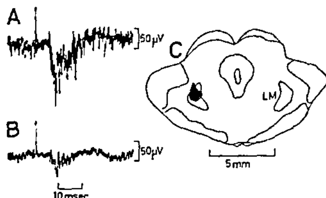


Fig 1 *Stereotactic lesion in the medial lemniscus* Correct placement of the electrode in the mesencepnalon was ascertained by recording the response evoked by electrical stimulation of the skin of the contralateral paw (A) The response was reduced (B) after the lesion indicated by the black area (C)

lemniscus as shown in the atlas of Jasper and Ajmone-Marsan (1960) A needle with a 1 mm bared tip was inserted to coordinates 4 mm frontal 5 mm lateral and 3 mm vertical In one animal the vertical coordinate was ascertained by recording the gross response evoked by electrical stimuli delivered to the contralateral forepaw (Tarnecki 1962) The response had a latency of 6 msec and followed the stimuli at 10 sec The amplitude of the response was 150 μV (Fig 1 A) before and about 50 μV (Fig 1 B) after the lesion was made The site and extent of the lesions were verified histologically

Testing Posture and movements of the freely moving cats were studied by photography and slow motion cinematography soon after recovery from anesthesia and once a week for one to 20 weeks after the operation (Table I) Flexor reflexes were elicited by strong stimuli pinching the pad, or by weak stimuli gentle stroking or by directing a jet of air on the paw The scratch reflex was elicited either as an unconditioned response to scratching of the skin around the ear or as an instrumental response to obtain food (Jankowska 1959)

To record muscle action potentials the cats were suspended in a sling with the extremities hanging freely

Initially the sling position is more disturbing to a cat than the position preferred by Liddell and Phillips (1944 with the rump in the observer s lap the hind limbs unsupported and the spine vertical This position however elicits tonic extension of both hind legs in normal cats Tonic extension of the legs struggling or signs of anxiety were absent in cats trained in the sling for an hour each day for about a week The recordings were taken without and often with light sedation 10 mg kg pentobarbital sodium Trains of electrical stimuli were applied through needle electrodes to the tibial nerve at the ankle at intervals of 5 sec (5 pulses of 0 1 msec duration at 250 sec The stimu

10 000 and 30 cycles sec The activity was recorded continuously on film moving at 2 5 mm sec from the face of a two beam oscilloscope the stimulating current was recorded on one beam as the voltage over a 100 ohm resistor in series with the stimulating electrodes

Histology The cats were perfused under pentobarbital sodium anesthesia with 10 % formalin in Ringer's solution Serial sections 20μ from the region of the lesion were stained by the Spielmeyer or Weil technique Sections from above and below the lesion in one animal (B Fig 2 were stained by the Marchi technique

Results

Neurological examination

I Cats with unilateral pyramidotomy Motor deficits involved the contralateral limbs and were not systematically different when the lesion destroyed one bulbar pyramid cleanly (A, Fig 2) or was incomplete in one pyramid (C, Fig 1) The signs gradually diminished and after one month the only abnormalities were weak flexor reflexes in contralateral legs and a slight delay in contact placing Cats with lesions involving

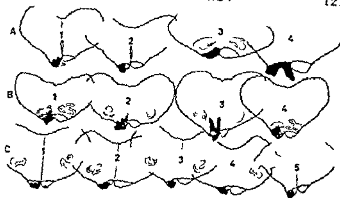


Fig 2 *Lateral pyramidal lesions in medulla oblongata* Absent or destroyed tissue is indicated in black In four cats (A) the lesions were complete without damage to other structures In 4 cats (B) the lesions were too large involving in addition the medial lemniscus and reticular formation In 5 cats (C) the pyramidal lesions were incomplete

the medial lemniscus and reticular formation as well as one pyramid (B, Fig 1) differed only with respect to the position of the paws at rest and during walking (see p 6)

Movements When struggling the cats used the affected limbs less often and less forcibly than the normal limbs When dropped from table height the cat usually regained its position normally, evidencing weakness or slipping of the hind leg on the affected side for no more than the first few postoperative days When grooming clawing, playing feeding and fighting, however they used the affected and normal paws alike One cat was trained for another purpose to obtain food by pressing a lever and chose to do it with the right paw before as well as after left pyramidotomy

Gait Stepping movements were alike on the two sides in seven cats Six cats showed slight abnormalities in gait the affected paws were not lifted from the floor during the flexion phase of the step and they sometimes slipped sideways Slipping was especially frequent when the cat walked along a narrow ledge or a ladder Marshall 1934) and the limb was then not replaced quickly or accurately This sign lasted one week after the operation

Posture The posture was usually normal both in the sitting and standing positions When the lesion involved the medial lemniscus the resting position of the affected limbs was sometimes awkward (illustrated in 1 Fig 3) This sign lasted one week after the operation

Placing reactions Visual placing was defective for the first postoperative days the paw contralateral to pyramidotomy was not placed or was placed later than the normal paw, even when the cat could see the edge of the table illustrated in 5 and 6 Fig 3) Defective contact placing was still present as long as the animals were followed (survival time given in Table 1 when their eyes were shielded the cats placed the affected paws with a delay

Extensor tone and flexor activity When the cat was suspended in a sling with the legs pendant the affected limbs hung in the extended position whereas the normal limbs were flexed (illustrated in 4 Fig 3 and in Fig 4 the difference between the sides being evident as long as the cats survived Table 1 Ther

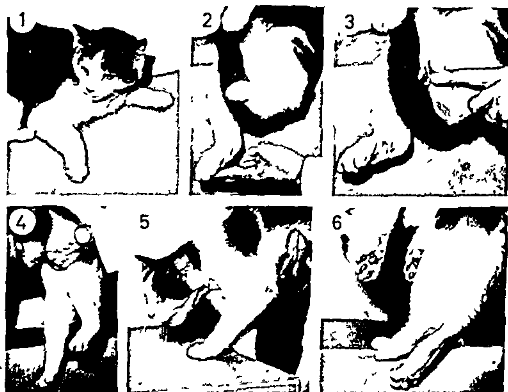
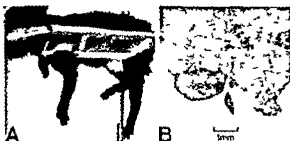


Fig. 3. Cat with pyramidotomy on the left side also involving the medial lemniscus (group B. Fig. 2) photographed 4 days postoperatively. 1. Awkward position of the right forepaw. 2. A flexion reflex in the right hind limb was not elicited by touch. 3. Normal flexion reflex in the left hindlimb. 4. Cat lifted off the ground: note extended position of right hindlimb. 5 and 6. Absence of visual placing reactions in right extremities.

in resistance to passive stretch of the limbs on the two sides. Supporting a paw yielded about the same resistance on both sides except that the normal leg was more often withdrawn when the cat struggled. A difference in resistance to passive flexion was sometimes felt when the cat was apprehensive; the difference disappeared, however, when the cat was further accustomed to the sling position, to the observer, and to pressure applied to the pad. Flexor reflexes, whether elicited by strong or weak stimuli, were weaker on the side contralateral to pyramidotomy, or they were absent. The difference between the two sides was most pronounced when the stimuli were weak (illustrated in 2 and 3, Fig. 3); it diminished gradually but lasted throughout the survival period.

Scratch reflex. This reflex could be elicited with about the same threshold on both sides after pyramidotomy. The initial movement of the scratch reflex, reinforced by food after conditioning (p. 119), was performed equally well before and three weeks after pyramidotomy (3 cats of group A).

Description of the lesions (Fig. 2). Group A. Four cats had complete unilateral lesions of the pyramids without damage to lemniscal or reticular fibres.



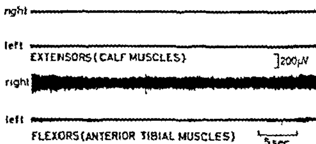


Fig 4 *Extended left limbs in an alert cat (A) 7 days after pyramidotomy (B) on the right side (Weil stain)* Action potentials were absent in extensor muscles The affected limbs hang low because flexor tonus normally elicited in this position (right side) was reduced

Group *B* In five cats the unilateral lesions were too large, involving part of the medial fillet and part of the reticular formation in addition to pyramidal fibres Ascending degeneration in the medial lemniscus was evident in the Marchi stained sections of cat B 2

Group *C* In four cats the lesions were too small, involving only a part of one pyramidal tract, other structures were not damaged Cat 1 had a small lesions of the contralateral pyramid

II Cats with unilateral medial lemniscotomy The lesions interrupted at least half of the fibres in each of the three cats (Fig 1 C) Complete interruption of the medial lemniscus was not attempted because it would involve a large part of the reticular formation *Neurological deficits were small* and lasted no more than one week Awkward positions of the extremities contralateral to the lesion were observed in all cats during stepping the affected limbs sometimes slipped on the floor There was a slight delay in the contact placing reaction When the cat was lifted from the ground the extremities of both sides struggled equally and had the same tendency to end in a flexed position The flexion reflex was the same on the two sides with both strong and weak stimuli

Muscle action potentials

I Cats with unilateral pyramidotomy When the cat was suspended in a sling with the legs hanging freely, activity in extensor muscles was absent on both sides and activity was present in the flexors on the n rmal side Fig 4 That the limbs contralat

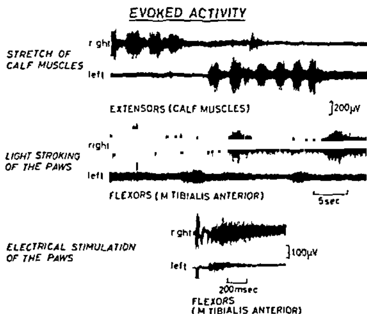


Fig 5 Action potentials recorded from flexors and extensors of the hind limbs 7 days after section of the right pyramid (same cat as Fig 4) ... [illegible]
lateral to pyramidotomy

to pyramidotomy hung lower than the normal legs was therefore due to decreased tonic flexor activity (Fig 4) Electrical activity induced by stretch was about the same in the calf muscles on both sides Light stroking of the dorsum of the paw or air jets directed at the paw elicited more muscle activity in the anterior tibial muscle of the ipsilateral than of the contralateral limb Similarly, trains of electrical stimuli of two times threshold strength applied close to the ankle nerves of the two sides elicited briefer and smaller bursts of action potentials on the contralateral than on the normal ipsilateral side The difference in response on the two sides was most pronounced during light sedation (Fig 5)

II Cats with unilateral medial lemniscotomy

When the cat was suspended in a sling with the legs pendant, flexor activity was equal on the two sides and extensor activity was absent Air jets directed at the paws elicited the same amount of activity in the anterior tibial muscles of the two sides

Discussion

The motor deficits produced by unilateral pyramidotomy in our cats corresponded closely to Marshall's 1934 description though the degree of involvement seems to have been less and recovery even faster in our animals than in his, and all the motor signs were contralateral Initially the gait could be abnormal the legs not being

lifted normally or slipping sideways Awkward positions of the paws could be uncorrected, the animals could fall to the affected side when pushed from a table and the hind leg could then slip sideways By confining lesions to the medial lemniscus we were able to show that these motor deficits were due to the lemniscal damage which often accompanies pyramidal section at the bulbar level and not to the pyramidotomy as such These signs lasted a few days to a week

Of the signs due to pyramidotomy, less use of the affected limbs in struggling difficulty in walking on a ladder and absent or delayed visual placing recovered within a week In agreement with Liddell and Phillips (1944), delayed contact placing and the extended position of the pendant legs were persistent for the survival period in our animals up to 5 months and in theirs to 12 months

At no time did we see the inertia in initiating phasic movements such as clawing or scratching which was like the other motor signs permanent in Tower's (1935) animals Indeed in our animals the scratch reflex to conditioned and unconditioned stimuli was elicited and executed normally on the two sides We are at a loss to explain the discrepancy between our Marshall's (1934) and Liddell and Phillips (1944) findings on the one hand and those of Tower (1935) on the other The only explanation we can offer is that her lesions were larger Some of our sections, though dividing the pyramid completely, were confined to it and our lesions placed in the medial lemniscus destroyed no more than half the fibres She describes her pyramidal lesions as being complete and as involving massive homolateral degeneration of the medial lemniscus

We cannot agree with Liddell and Phillips (1944) that extensor hypertonia accounts for the extended position of the pendant limbs They measured the pressure which had to be applied to the pad to cause the leg to flex since it was consistently higher contralateral to pyramidotomy (sometimes exceeding the body weight of the animal), they concluded that extensor tone was increased by pyramidotomy We interpret the difference they found as due to a normal flexion reflex on the ipsilateral side elicited on the background of tonic extension of both hindlegs normally present in cats held as described by Liddell and Phillips (1944) (p 120) By recording the muscle action potentials from flexors and extensors of the leg we were able to confirm Tower's conclusion based on her observation of the motor deficits that the primary disorder after pyramidotomy involves flexion We found that the extended position of the pendant leg is due to inactivity of the flexors and that the flexors activated electrically through the spinal reflex pathway responded less briskly on the affected than on the normal side The activity recorded in the extensors in response to stretch was the same on the two sides This effect of pyramidotomy is in agreement with stimulation experiments pyramidal volleys elicited flexion movements and inhibited extensor activity (Kato Takamura and Fujimori 1964 Laursen and Wiesendanger 1966)

The work was supported by the Swiss N[illegible]nal Founda[illegible]n f[illegible]r Scientific Research (Grant No 2903 and the Danish State Research F[illegible]n[illegible]a[illegible]n

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Acta physiol. scand. 1966. 68. 127—128

From the Institute of Anatomy and Histology, University of Lund Sweden

Adrenergic Nerves in Spinal Ganglia of the Cat

By

CHRISTER OWMAN and MAURIZIO SANTINI[1]

Adrenergic nerve fibres are present in the feline Gasserian ganglion in which they are related to both the ganglion cells and blood-vessels (Santini 1966). These findings prompted similar studies on other sensory ganglia, on which investigations are in progress. The present report gives preliminary data showing that adrenergic nerves occur in spinal ganglia of the cat.

Five cats were killed, and spinal ganglia from the cervical, thoracic, lumbar, and sacral levels were dissected out bilaterally. They were treated according to the method of Falck and Hillarp for the fluorescence microscopic visualization of adrenergic nerves (see Falck and Owman 1965). Under the conditions used the catecholamines are transformed into fluorophores emitting an intense green light.

Nerve cells with a specific amine fluorescence were not found in the ganglia. The cells varied considerably in size, and contained a varying amount of yellow-brown autofluorescent granules in the cytoplasm (Fig. 1).

Immediately outside the ganglion, bundles of green fluorescent axons of both the smooth and varicose type were seen in the fascicles of myelinated nerves. At the ganglion border the fluorescent bundles split up into smaller branches. Delicate varicose terminals, having the fluorescence characteristics of primary catecholamines, coursed isolated in various directions among the myelinated nerves. Many, if not all, of these adrenergic fibres could be directly related to blood vessels.

In the thoracic ganglia a moderate number of varicose nerve terminals, often single, coursed among the ganglion cells (Fig. 1). Some fibres ran contiguous to and encircled the perikarya — usually those of larger size — in a manner suggestive of a synaptic arrangement (Fig. 1). Other nerve fibres enclosed small blood vessels. Several isolated varicose axons could be followed for a considerable length in between the ganglion cells. The number of intraganglionic adrenergic nerve terminals was largest in the thoracic ganglia. Fewer nerves were present at the cervical level and only scattered fibres occurred in the lumbar and sacral ganglia. A relation of adrenergic nerve terminals with non fluorescent ganglion cells could be demonstrated at all levels studied.

The origin of the adrenergic fibres in these ganglia is as yet unknown. They may arise from the sympathetic chain: cervical sympathectomy causes a severe reduction

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Fig 1 Fluorescence micrograph of spinal ganglion from the thoracic level All fluorescence within perikarya is autofluorescence Left Survey picture Green fluorescent nerve terminals run between ganglion cells and in bundles of myelinated axons 70 × Right Varicose adrenergic nerve terminal encircling large ganglion cell in a synaptic manner 400 ×

in the amount of green-fluorescent fibres within the Gasserian ganglion of the cat (Kågstrom, Owman and Santini, unpublished observations) The possibility of a central origin should also be considered (*cf* Mitchell 1953, Dahlström and Fuxe 1965) Experiments are in progress to obtain information on this problem

Supported by the Swedish Medical Research Council (grants No 12X 712-01 and 14X 732-01)

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Acta physiol. scand. 1966. 68. 129—133

From the Department of Physiology, Karolinska Institutet, Stockholm Sweden

The Effect of Cold Exposure on the Catecholamine Excretion of Rats Treated with Decaborane

By

D G JOHNSON[1]

Received 25 November 1965

Abstract

JOHNSON, D G *The effect of cold exposure on the catecholamine excretion of rats treated with decaborane* Acta physiol scand 1966 *68* 129—133

Rats treated with decaborane exhibited a marked reduction in the noradrenaline (NA) content of heart liver and spleen but no significant fall in adrenal catecholamines Decaborane treated animals excreted less NA but more adrenaline (A) than control animals When exposed to 3° C control animals survived and showed increased NA excretion in contrast to decaborane treated rats which however responded with increased excretion of A until death after 4—6 days Unlike adrenalectomized controls adrenalectomized rats treated with decaborane failed to increase their NA [illegible]

app[illegible]

mec[illegible]

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Studies of urinary excretion of catecholamines in man and animals exposed to cold (Arnett and Watts 1960, Cottle 1960, LeBlanc and Nadeau 1961, Leduc 1961) have drawn attention to the important role of both the adrenergic nervous system and the adrenal glands in cold adaptation and survival It has also been shown that catecholamine-depleting drugs such as reserpine prevent the normal physiological response to cold, leading to poor adaptation and death (Dandiya, Johnson and Sellers 1960, Hoffman 1959, Zilberstein 1960, Taylor 1961) The failing NA excretion on exposure to cold also suggests inhibition of NA synthesis by reserpine (Leduc 1961, Johnson 1963)

Decaborane has been found to deplete both the brain (Merritt, Schultz and Wykes 1964) and peripheral organs (Euler and Lishajko 1965) of NA The purpose of this study was to determine whether an increased adrenergic activity can be elicited when tissue catecholamines are depleted by decaborane In addition it was thought that further evidence might be obtained for the role of the adrenergic nervous system and the adrenal medulla in cold adaptation

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TABLE I Catecholamine content of organs of control rats and rats treated with decaborane Mean ± SEM

Organs	Control rats		Decaborane treated	
	NA	A	NA	A
Heart ng/g	350 ± 28	8 2 ± 3 8	47 7 ± 3 1	7 5 ± 1 9
Spleen ng/g	331 ± 22	9 8 ± 4 0	36 2 ± 14 1	3 6 ± 3 6
Liver ng g	33 1 ± 2 6	1 0 ± 0 3	6 1 ± 1 5	1 6 ± 0 8
Adrenals µg/kg BW	26 0 ± 6 5	102 ± 8	24 1 ± 9 0	89 3 ± 18 6

Methods

All experiments were carried out on male rats (300—350 g) of the Sprague-Dawley strain The [illegible]

[illegible]

funnels and a glass trap system for the separation of feces The urine was adjusted to pH 3—4 by [illegible]

[illegible] was dissolv- [illegible] 12 mg ml [illegible] succeeding alternate days [illegible] Organ determinations were made after 6 days of this treatment

Results

The doses of decaborane used in these experiments resulted in occasional mortality among rats kept at 20 C (15 % of the rats were dead at the end of 1 week of treatment) Adrenalectomy did not significantly affect the mortality after decaborane treatment All of the rats receiving decaborane became lethargic and sedated, but could easily be aroused by handling Rats treated with decaborane lost appetite and showed an average weight loss of 15 g per week

Organ catecholamines Decaborane caused a marked reduction in the NA content of heart, spleen, and liver Table I After 6 days of treatment with decaborane 3 injections) the NA content of the heart and spleen was less than 14 % of control levels, while the liver contained less than 19 % of control levels The A content appeared to be little changed, except for an insignificant decrease in the spleen The adrenal glands showed no decrease in NA content and only a slight insignificant decrease in A

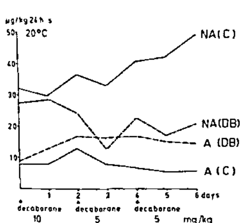


Fig 1

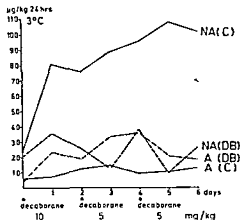


Fig 2

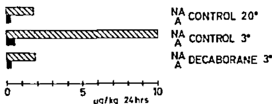


Fig 3 Effect of decaborane (10 mg/kg) on the urinary excretion of catecholamines from adrenalectomized rats exposed to 3° C 6 rats per group The decaborane treated animals died within 20 hrs

Urinary catecholamine excretion The urinary excretion of NA in rats treated with decaborane and placed at 20° C decreased over the course of 3 days to about one half of normal values (Fig 1) In the same time period the A excretion increased to approximately twice normal levels The urinary levels of NA and A for control and decaborane-treated rats placed at 3 C are shown in Fig 2 The control rats increased their NA excretion in the first 24 hrs and maintained this for the duration of the experiment The control A values increased to twice the value obtained at 20° The decaborane treated rats did not increase their NA excretion The NA levels rose slightly the first day, followed by a decrease to levels below that which was normally excreted at room temperature The A levels of decaborane treated animals rose markedly over the course of 4 days, whereafter the decaborane treated rats became moribund, and the urine volumes as well as the A excretion tended to fall

All of the control animals survived the exposure to 3° C, whereas none of the decaborane-treated animals lived more than 6 days

Adrenalectomy In the experiment with adrenalectomized rats at 3° C the control animals responded with a marked increase in NA levels similar to those of non-adrenalectomized rats (Fig 3) The A excretion of the adrenalectomized controls rose to a level similar to that excreted by non-adrenalectomized rats at room temperature Decaborane-treated animals failed to increase their NA excretion when placed at 3° C Their excretion of A also failed to rise, and they died in less than 20 hrs No adrenalectomized control animals died at 3° C

Discussion

The decrease in tissue NA content after decaborane treatment agrees with the results obtained by other workers (Merritt *et al* 1964, Euler and Lishajko 1965) The decrease to levels below 20 % in the liver, spleen, and heart compared to the decrease to 40 % observed in the brains of rats given similar doses of decaborane by Merritt *et al* (1964) suggests some barrier to decaborane absorption in the brain or that the brain catecholamine-containing neurons are less sensitive to decaborane The slight effect on the adrenal glands is also conspicuous

The finding that NA excretion in urine only decreases to 50 % of control levels when the organ content of NA is decreased to much lower levels could be due to changes in the per cent of released NA which is excreted in the urine, or it could indicate that despite severe depletion the NA remaining in the adrenergic neurons is still available for a certain minimal level of activity This observation agrees with the evidence obtained from simultaneous studies of urinary excretion and tissue content of catecholamines after reserpine (Leduc 1961) The rise in A excretion of rats treated with decaborane may reflect a direct releasing effect of decaborane on the adrenal gland

The failure of the decaborane-treated rats to increase NA excretion in response to the cold stress suggests a diminished supply of transmitter, presumably owing to deficient biosynthesis of NA[1]

It is known that A is released in animals subjected to severe cold exposure (Hartman *et al* 1923, Cannon *et al* 1927, Saito 1928 Wada Seo and Abe 1935) However, as shown by later workers (Heroux 1955, Desmarais 1957, Leduc 1961) adrenalectomy *does not prevent animals from adapting and surviving cold* exposure On the other hand Hoffman (1959) noted a complete loss of thermoregulation in adrenalectomized animals after treatment with reserpine and exposure to cold, with a certain degree of correction with A However Sellers *et al* (1951) were unable to artificially acclimate clipped rats to cold with A Because of the apparently slight effect of decaborane on the adrenomedullary stores of catecholamines found in this study, the use of decaborane seemed to provide an unusual opportunity to study the response of this organ to cold while at the same time selectively suppressing the adrenergic nervous system The comparatively large increase in A excretion in the

[1] *Addition to proof* Merritt and Schultz (1966) have recently produced evidence to show that decaborane selectively inhibits the decarboxylation of dopa

urine of decaborane treated rats placed at 3° C and the rapidly occurring death of adrenalectomized animals treated with decaborane and exposed to cold seem to support the idea of A release as a functionally important physiological response to cold rather than just a coincident alteration in an animal unable to adapt Assuming a recovery in the urine of 4 % of the A secreted by the adrenal glands (see Leduc 1961) the decaborane treated rats exposed to 3° C increased their daily secretion of A to amounts equivalent to the entire A content of the adrenal glands This high release rate implies a considerable associated rate of synthesis, which can be taken as further evidence of the relatively slight effect of decaborane on the adrenal gland The death of the decaborane treated rats exposed to cold after approximately 4—6 days suggests that A release provides only partial or temporary protection

The research reported in this document has been sponsored by the *AIR FORCE OFFICE OF SCIENTIFIC RESEARCH* under grant AF EOAR 66-7 through the European Office of Aerospace (OAR) United States Air Force

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Acta physiol. scand. 1966. 68. 134—140

From the Department of Physiology, Karolinska Institutet Stockholm Sweden

Neurotransmitter Deficiency and Reloading in Noradrenaline Depleted Rabbits

By

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Received 7 March 1966

Abstract

BYGDEMAN, S and U S VON EULER *Neurotransmitter deficiency and reloading in noradrenaline depleted rabbits* Acta physiol scand 1966 *68* 134—140

The vasoconstrictor effect of intermittent lumbar sympathetic stimulation of the hindleg of the rabbit pretreated with noradrenaline (NA) depleting doses of decaborane or prenylamine rapidly subsides in contrast to untreated controls When the interval between the trains of stimuli is increased a partial recovery of the stimulation response is observed suggesting an accumulation of transmitter probably as a result of continuous resynthesis Injection of NA (1—5 µg/kg) or dopamine (50—100 µg kg) also causes an enhanced stimulus response suggesting uptake of NA and increased rate of synthesis respectively An increase in stimulation response was observed after administration of dopa in prenylamine-treated but not in decaborane-depleted animals

Under physiological conditions replenishment of the adrenergic neurotransmitter at the site of release occurs at such a high rate that no functional deficiency can be observed even during prolonged activity In agreement with this no net loss of noradrenaline NA in the spleen of the cat was observed after stimulation of the splenic nerves for 10—15 min except in one animal in bad condition Euler and Hellner Bjorkman 1955, More recently Dearnaley and Geffen (1966) have shown that on electric stimulation of the splenic nerve in cats for 1/2—5 min the neurotransmitter content in the stimulated part of the spleen decreased only by 7 7 per cent in comparison to an unstimulated part calculated on the DNA content of the tissue However, after prolonged and intensive electrical stimulation of the sympathetic chain of the cat Kernell and Sedvall 1964 succeeded in causing a considerable decrease of the NA stores in the gastrocnemic muscle

After depletion of the NA stores in the cat with reserpine to such an extent that electrical stimulation of the lumbar sympathetic did not longer evoke vasoconstriction in the perfused leg it has been possible to restitute a vascular response by administration of NA or A Burn and Rand 1958, Rosell and Sedvall 1961, This effect was of relatively short duration, indicating that the amount of NA taken up and available for transmitter purposes rapidly diminished during stimulation

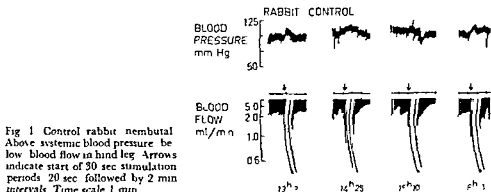


Fig 1 Control rabbit nembutal Above systemic blood pressure below blood flow in hind leg Arrows indicate start of 30 sec stimulation periods 20 sec followed by 2 min *intervals Time scale 1 min*

It therefore seemed of interest to study restitution of neurotransmission after depletion of NA by decaborane (Merritt, Schultz and Wykes 1964) and prenylamine (Segontin[R]) (Schone and Lindner 1962)

It has recently been demonstrated that after depletion of the NA stores in the rabbit with prenylamine or decaborane, administration of NA or A is followed by an uptake into the stores to normal values (Mackenna 1965, Euler and Lishajko 1965 a) Moreover, it was found that refilling of the heart after depletion with prenylamine could be effected also by some NA precursors (tyrosine, dopa and dopamine) or to some extent even by the action of other amines (phenylethylamine, tyramine and octopamine) (Euler and Lishajko 1965 b) After decaborane depletion, dopamine but not dopa caused refilling of the heart (Euler and Lishajko, unpubl obs) It therefore seemed of interest to find out whether various precursors could restore transmission in a NA depleted system

Methods

Rabbits 2—3.5 kg [illegible]
[illegible]
doses of 2 mg/kg at 1/2 hr intervals

The animals were anesthetized with nembutal 20—40 mg/kg i v or in some cases with urethane 1.3 g per kg i v The blood flow [illegible]
femoral ar[illegible]
a Grass p[illegible]
artery by [illegible]
vein

The lumbar sympathetic chain was tied off at the level of the renal hilus, and bipolar platinum electrodes applied at the nearest distal interganglionary segment Supramaximal stimuli of 10 msec duration 20 per sec were delivered for 30 or 60 sec with intervals of 1 or 2 min by means of an automatic device (Perman and Persson 1962) Untreated controls were prepared and stimulated in the same way

Results

Untreated animals

In 3 animals the lumbar sympathetic was stimulated for 30 sec with 2 min interval for several hours As shown in Fig 1 the response is not perceptibly changed in the 4 stimulation periods between 13th and 16th hours

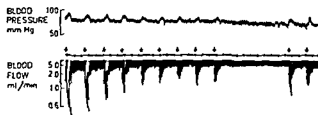


Fig 2 Rabbit decaborane 4 + 2 mg/kg 48 and 24 hrs previously Declining vasoconstrictor response to 30 sec stimulation periods 20 sec Intervals 2 min Increased response after stimulus free interval Time 1 min

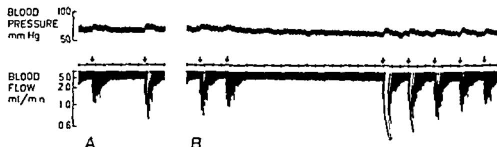


Fig 3 Rabbit decaborane 4 + 2 mg/kg 48 and 24 hrs previously Increased response after exclusion of 1 (A) and 5 (B) stimulation periods of 60 sec 20/sec Intervals 2 min Time 1 min

Decaborane depleted animals

The decaborane-treated animals had a characteristic sedated appearance with closed eyelids and few spontaneous movements Their blood pressure was usually normal or slightly lower than normal at the beginning of the experiment with a tendency to fall gradually over the time course of the experiments

The initial 30 sec nerve stimulations (600 stimuli) strongly reduced the blood flow through the leg as in the controls However, in contrast to the untreated animal the vasoconstrictor effect gradually diminished in the decaborane treated animal The rate of deterioration of the effect varied in different animals, presumably depending on the degree of decaborane action Generally a definite decrease in response could be seen after 10—20 stimulation periods (Fig 2) If one or more stimulation periods were omitted, the response to the following stimulation period was markedly increased usually more so the longer the period of neuronal rest (*Fig 2 and 3*) This increase was of short duration and the pre rest response was again obtained during the 2nd to 4th post rest stimulation periods

On repeated stimulation the decreasing response finally reached a certain low level which was maintained as long as stimulation periods and intervals were unaltered, as illustrated in Fig 2 If during this stage stimulation was continued for 1 min or longer the vasoconstrictor response gradually disappeared suggesting complete temporary depletion of the available stores After a 1 2 min rest the response was restored to the same extent as before the previous stimulation

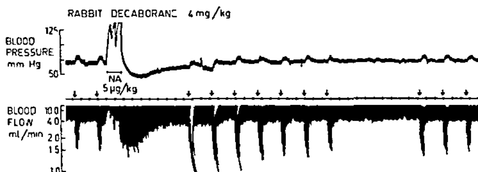


Fig 4 Rabbit, decaborane, 4 mg/kg 24 hrs previously Increased response to 30 sec stimulation periods 20 sec, after NA 5 µg/kg i v Intervals 2 min Time 1 min

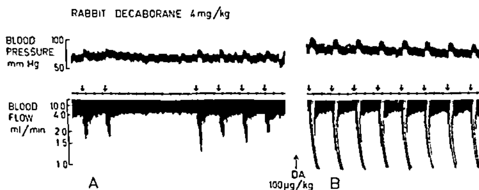


Fig 5 Rabbit, decaborane, 4 mg/kg i p 24 hrs previously Increased stimulation response following neuronal rest (A) and 100 µg dopamine per kg i v (B) Stimulation periods 30 sec 20 sec Intervals 2 min Time 1 min

Stimulation response after injection of NA

The effect of i v injection of NA was conspicuous (Fig 4) The smallest effective dose was 1—2 µg per kg With doses of 5—10 µg/kg the effect was usually quite marked and lasted for 5-10 subsequent stimulation periods, indicating an uptake which could be utilized for neurotransmitter functions

Stimulation response after injection of NA precursors and congeners

Dopamine in a dose of 100 µg/kg i v was found to produce a definite enhancement of the vasoconstrictor response to nerve stimulation Fig 5) lasting for a considerable number of stimulation periods Dopa or tyrosine, on the other hand in a dose of 10 mg/kg was without noticeable effect, suggesting that these compounds, if taken up by the synthesizing apparatus were not efficiently transformed to the transmitter under the prevailing conditions No definite effect could be detected after isoprenaline 28 µg kg, tyramine 0 5 mg kg and octopamine 25 µg kg

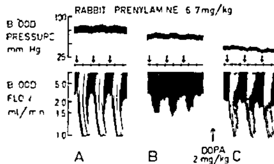


Fig 6 Rabbit prenylamine, 6 7 mg kg i v Stimulation period 60 sec 20 sec Intervals 1 min A Beginning of stimulation B After about 1 hr C 5 min after dopa 2 mg/kg i v

Prenylamine-depleted animals

Depletion after prenylamine given i v may be quite rapid as observed for the rabbit heart by Mackenna (1965) Thus the stores in the heart are largely depleted 1/2—1 hr after injection

The vasoconstrictor response to lumbar sympathetic stimulation gradually diminished in a similar way as after decaborane when stimuli were applied 1/2 hr after the end of the infusion of prenylamine The restoring effect of neuronal rest was also noted in the prenylamine-treated animals, although it was usually less marked than in the animals which had received decaborane

NA 2—5 μg kg i v also restored the vasoconstrictor effect to some extent as did dopamine 100 μg kg, although the effects were generally less conspicuous than in decaborane-treated animals A marked effect on the stimulus response was observed even after dopa 2 mg/kg (Fig 6) in contrast to the observations in decaborane-treated animals

Discussion

The experiments described in the present paper confirm in general the effects first noted by Burn and Rand (1958) and by Rosell and Sedvall (1961) in animals whose NA-stores had been depleted by reserpine The same general features were observed in the decaborane and prenylamine-depleted rabbits which consisted of a gradual diminution of the response to vasoconstrictor stimulation and partial recovery after administration of NA

In some respects our experiments have extended the observations made previously Thus a partial recovery of the response was regularly seen after increasing the interval between the trains of stimuli This effect suggests that the amount of transmitter available has increased during the prolonged interval

Previous experiments have shown that exogenous NA is rapidly distributed between granular and extragranular pools in the decaborane-depleted heart (Euler and Lishajko 1965 a) Thus redistribution between pools is less likely to be the underlying reason for the slow and gradual recovery on prolongation of the interval between stimulation It appears more probable that both the weak residual effect on intermittent stimulation (Fig 2 and the partial recovery following "neuronal rest" depend on resynthesis occurring at a slow approximately steady rate The amount

of NA available for immediate use in stimulation would then be a function of the interval between two consecutive trains of stimuli This interpretation does not, of course, exclude uptake as an auxiliary mechanism for the accumulation of trans mitter The recovery phenomenon was less marked in those animals in which the response to stimulation diminished rapidly which was often the case in the prenyl-amine treated animals suggesting a less efficient resynthesis

It is of interest to note that in reserpine depleted animals Rosell and Sedvall (1962) did not observe spontaneous recovery after disappearance of the response to stimulation, even after prolonged stimulus free intervals This seems to indicate that spontaneous resynthesis is impaired to a greater extent in their system Infusion of moderate amounts of NA restored the stimulation response as did dopamine and dopa after reserpine

No certain connection could be noted between height of blood pressure, blood flow and the appearance of the recovery after 'neuronal rest' The importance of the flow for the vasoconstrictor response has been emphasized by Folkow (1962) The term 'neuronal rest' was used by Brown, Davies and Ferry (1961) in a somewhat different context and was intended to signify the long rest necessary for removal of transmitter from the receptor In the present report the expression is used to re present the time interval necessary for accumulation of an extra amount of transmitter This amount was small as suggested by its rapid disappearance following new trains of stimuli

The partial recovery of the response after 'neuronal rest" had to be considered in the "reloading' experiments with NA or its precursors, in which it was often necessary to increase the interval after the injection of the substances

In view of the positive results on the recovery of the vasoconstrictor response observed by Rosell and Sedvall (1961) in reserpinized cats, it might be specially mentioned that dopa, in contrast to dopamine, was not effective in decaborane-treated animals, in doses up to 10 mg/kg

According to Merritt and Schultz (1966) decaborane exerts a blocking effect on the decarboxylation of dopa Thus might be a major contributing factor to the failure of dopa to restore the vasoconstrictor response in decaborane-depleted animals but not in reserpine treated ones Dopa or tyrosine in doses of 50 mg/kg were also with out effect on the NA content of the heart in decaborane-depleted rabbits (Euler and Lishajko, unpublished experiments), in contrast to prenylamine-depleted animals As shown in the present study dopa was effective in restoring the vaso-constrictor response in prenylamine treated animals

The experiments reported here give no information as to the relationships be tween neurotransmitter action and the amounts of transmitter present in the organ However the depletion observed in various organs after decaborane or prenyl amine makes it appear likely that the transmission failure is due to depletion of the transmitter The constant low level of response to stimulation in the depleted animals as well as the partial recovery after neuronal rest supports the concept of a conti nuous slow resynthesis under these conditions suffi [illegible]nt to [illegible]

of transmitter during each stimulation period in depleted animals The full response in the control animals speaks in favour of a rapid and efficient resynthesis which is in agreement with the findings of less then 10 per cent loss of transmitter in the organ after up to 5 min uninterrupted stimulation of the splenic nerves in the cat (Dearnaley and Geffen 1966) In the experiments of Euler and Hellner-Björkman (1955) it was observed that in one cat with low blood pressure and low initial NA values of the spleen 20 min nerve stimulation caused a 50 per cent fall in the NA content of the stimulated part In this case the low control values may be indicative of a low synthesis rate

This investigation was supported by grants from the Swedish Medical Research Council under project no 14X 97 02 the Gustaf and Tyra Svensson Foundation (U S v E) and the Swedish National Association for Heart and Chest Diseases (S B)

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Acta physiol. scand. 1966. 68. 141—151

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Intestinal Dipeptidases

Development of Dipeptidase Activity in the Small Intestine of the Rat as Related to the Development of the Intestinal Mucosa

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Received 7 March 1966

Abstract

LINDBERG, T and CH OWMAN *Intestinal dipeptidases Development of dipeptidase activity in the small intestine of the rat as related to the development of the intestinal mucosa* Acta physiol scand 1966 68 141—151

The development of five intestinal dipeptidase activities (L alanyl L-glutamic acid L alanyl L proline, glycylglycine glycyl-L-leucine and glycyl L-valine dipeptidase activity) in the rat from 15 days *post coitum* up to 3 weeks *post partum* was investigated and related to the structural development of the rat small intestinal mucosa Very low enzyme activities were found in the period of mucosal cell proliferation (15 to 18 days *post coitum*) At 18 1/2 days *post coitum* the period of mucosal cell differentiation was commencing and simultaneously the dipeptidase activities began increasing to reach maximal values at parturition by which time the differentiation was finishing Within a few hours of birth when the newborns had suckled an abrupt and significant fall to the relatively low adult level was observed for four of the dipeptidase activities The fifth enzyme activity (L-alanyl L-proline dipeptidase) declined more slowly postnatally and first reached the relatively low adult level during the 3rd week *post partum* The relation of the changes in the intestinal dipeptidase activities to the structural characteristics of the mucosa during the development has been discussed

In man the intestinal dipeptidase activities are present as early as 11 weeks of fetal age (Lindberg 1966 a) At this stage, the enzyme activities in the small intestine are of about the same magnitude as in adults (Lindberg 1966 b), and furthermore the mucosa of the small intestine in human fetuses is already rather well differentiated (Johnson 1910, Patzelt 1931) Therefore, it seems necessary to investigate intestines from still earlier stages of development However since adequate amounts of human fetal material could be obtained only at the period between 11 and 23 weeks of fetal age, an investigation was performed on developing rats In the present report the dipeptidase activities of the small intestine were related to the structural development of the small intestinal mucosa at frequent intervals between 15 and 22 days *post coitum* Further similar studies were performed at various postnatal stages up to 3 weeks *post partum*

Material and Methods

Animals A total of 2?2 fetuses were obtained from 35 albino rats weighing 150—200 g In these

was assigned as day 1 post coitum In the strain of rats used the length of the gestation period is ... to 22 1/2 days *post coitum* — At the appropriate stage the mother animal was decapitated under light ethyl ether anesthesia and the fetuses removed for enzyme studies and histological analysis Those used for the enzyme studies were immediately put in a cold box (4°)

Newborn *rats from 6 litters were taken immediately after delivery, before suckling From 3* litters rats were also taken about 6 hrs after birth, these rats had thus sucked for a couple of hours

part below the diaphragm in the older fetuses) at 5μ in the sagittal plane Staining was performed alternately in hematoxylin-eosin McManus periodic acid Schiff (PAS) (Pearse 1960) and in Mallory's phosphotungstic acid hematoxylin (McManus and Mowry 1960) The preparations were mounted in Permount (Fischer)

d in the cold (4°) microscope The length etal age In the younger littermates were pooled

In the older fetuses (19—22 days *post coitum*) the small intestines were divided in two or three parts

small intestine failed however and various amounts of colostrum was left

In the older animals (from 3 days of life) the small intestine was slit longitudinally and the mucosa carefully cleaned by a dry soft cloth The whole proximal middle and distal 10 cm of the small intestine was taken and cut into pieces

The separate preparations were homogenized in 1 ml (fetal material) or 2 ml 0 1 M NaCl in an icebath for 2 min at 14 500 rpm (MSL-homogenizer) After standing 30 min the homogenates were centrifuged (International model HR 1 27 000 g 30 min) After suitable dilution with redistilled water according to total nitrogen content the clear supernatants were used directly as the enzyme solution

Assays The dipeptidase activities were determined by the spectrophotometric method as previously de ... ons were the same as

Protein

(Armour

culated as 17 per cent of the protein content

Unit of dipeptidase activity One unit is defined as the activity hydrolyzing 1 μmole of dipeptide per min at 40° (Josefsson and Lindberg 196? b

Results

Development of small intestine

At the earliest stage analyzed 15 days *post coitum*) the stratified epithelium (Fig 1A) lining the simple lumen of the intestinal anlage is about 30 μ thick, consisting of low columnar cells arranged in 2 to 3 layers The oval nucleus occupies a considerable

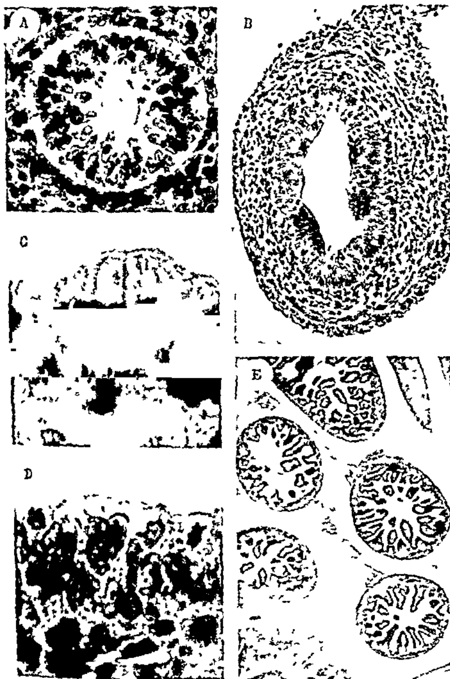


Fig 1 M crophotographs demonstrat ng erta n characterist s lur ng devel p e t rat sn all intestine

(A) 15 days *post coitum* Undifferen e l est nal anlage w h strat fied ep l el u Hematox ylin-eos n 480

(B 18 days *post coitum* Early s s f v llus f rmat on in n ddle p rt f small intestine Intraepithelial vacuole in left upper par M ll rs staining 190

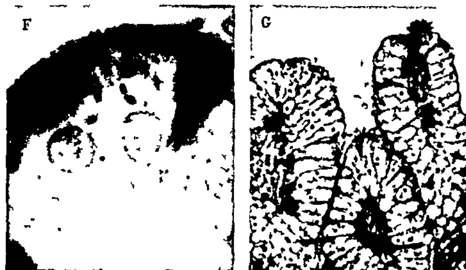


Fig 1 cont

(C) 18 1/2 days *post coitum* Brush border at the surface of the simple columnar epithelium of jejunum Hematoxylin eosin 1600 ×

(D) 18 1/2 days *post coitum* Brush border at the surface of the stratified epithelium of ileum Hematoxylin-eosin 1025 ×

(E) 20 days *post coitum* Survey picture showing well developed villi in jejunum Mallory staining 40

(F 22 days *post coitum* PAS positive inclusions in the supranuclear cytoplasm in one of the cells around a vacuole of cells in the distal small intestine Goblet cell (black) to the right PAS 1400

G 3 weeks *post partum* Entire supranuclear cytoplasm of columnar cells in distal ileum is occupied by a single large vacuole containing a lot of rounded PAS positive (black) inclusions PAS 240

portion of the cell Large numbers of mitoses are collected along the luminal border The thickness of the epithelium has increased by about 10 μ in the following stage (16 days) owing to increasing size of the columnar cells At 17 days the cells of the 2 to 3 layers are arranged more irregularly The thickness of the epithelium is again about 30 μ Numerous mitoses are still present along the luminal border

After this period of cell proliferation differentiation of the intestinal epithelium commences Thus in the 18 day fetuses primitive villi begin to appear (Fig 1B) Proximally in the small intestine the epithelium forms longitudinally running ridges by thickenings consisting of up to 5 cell layers on top of a low mesenchymal core Between the villus formations the stratified epithelium is only 2 layered Intraepithelial vacuolization is a characteristic feature Distally, the development is less advanced the longitudinal ridges are barely recognizeable and consists only of slight epithelial thickenings Mitoses occur mainly within the thickened parts of the epithelium along the whole small intestine At 18 1 2 days the ridges are more prominent in the distal portion of the small intestine due to the ingrowth of a low broad based mesenchymal core underneath the epithelium Proximally the villi are well developed and covered by a simple columnar epithelium At this stage a delicate brush border has formed at the luminal surface of the columnar cells

throughout the small intestine (Fig 1C and D) In the next stage (19 days) no prominent change is seen in the appearance of the intestinal anlage

In fetuses 20 days old, prominent villi are found in the whole jejunum ileum (Fig 1E) The surface of the simple columnar epithelium is characteristically convex and the oval nucleus is quite large Mitoses are now relatively few and mainly collected in the intervillous parts Typical goblet cells have begun to appear within the epithelium and increase in number in the following stages

From the 21 day stage on the columnar cells of the epithelium are higher The luminal surface is flattened rather than convex, and covered by a well developed brush border The round nucleus is located in the middle or basal third of the cell Distally in the small intestine the supranuclear zone of the columnar cells often contain a small number of PAS positive granules of about 1 μ in diameter see further Owman 1963 1964 a and b) At 22 days *post coitum* the intestinal epithelium has an appearance similar to that in the previous stage However the supranuclear portion of the cells in the distal ileum is as a rule occupied by a large vacuole and the PAS positive inclusions (1—2 μ in diameter) are then often found around its periphery (Fig 1F) The first sign of the crypts of Lieberkuhn is seen at this stage as a narrow epithelial invagination into the underlying connective tissue between the villi

In the postnatal stages studied the development of the crypts of Lieberkuhn proceeds more rapidly in the proximal than in the distal small intestine At 3 weeks *post partum* both crypts and villi are well developed The mitotic figures present are mostly located in the crypts

In the specimens taken immediately after birth before sucking the simple columnar epithelium lining the villi of the ileum has an appearance similar to that in fetuses 22 days old The cellular inclusions have increased somewhat in number and tend to coalesce into small groups in the cleared supranuclear cytoplasm About 6 hrs later when sucking has begun the cell nucleus is pushed towards the base of the cell and the supranuclear cytoplasm especially near the nucleus now contains a large number of irregularly formed PAS positive inclusions of 1—3 μ or more in diameter During the next 2 days the amount and size of the inclusions increase considerably At 2 weeks after birth the whole supranuclear cytoplasm is occupied by a small number of very large PAS positive globules measuring up to 10 6 μ the nucleus is pressed basally At 3 weeks (Fig 1G) the general amount of inclusions now about 4 μ in diameter) is smaller and instead the entire supranuclear cytoplasm is present as a single large vacuole that compresses the nucleus towards the cell base — Very little inclusion material is found in the proximal portion of the small intestine at these stages

Development of dipeptidase activity

The assays of the various dipeptidase activities were performed at the respective pH-optimum (see below) The glycylglycine dipeptidase activity was determined in the presence of 5 mμmoles Co^{2+} ions (final concentration 2 95 × 10^{-4} M)



Fig 1 cont

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(D) 18 1/2 days *post coitum* Brush border at the surface of the stratified epithelium of ileum. Hematoxylin-eosin 1025

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Development of dipeptidase activity

The assays of the various dipeptidase activities were performed at the respective pH-optimum (see below) The glycylglycine dipeptidase activity was determined in the presence of 5 mμmoles

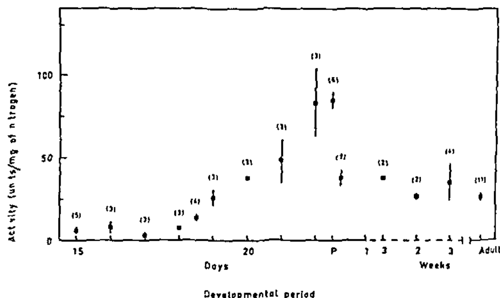


Fig 2 Pre- and postnatal development of glycyl L-leucine dipeptidase activity (units per mg nitrogen mean values ± S E M) in the rat small intestine The S E M for 18 days *post coitum* is ± 0 4 for 20 days *post coitum* ± 0 9 and for 3 days *post partum* ± 0 3 (bars covered by symbols) Figures in parenthesis indicates number of litters P – partus Optimum pH and 40

The results for glycyl-L-leucine dipeptidase activity are shown in Fig 2, in which the mean values and the standard error of the mean (S E M) of the activity, expressed as units per mg nitrogen present in the enzyme solution, are given for the various litters from the different stages of the development For the older fetuses as well as for the newborn rats and the sucklings, where various parts of the intestine were studied, the figures given are mean values calculated from the activities in the different intestinal portions The values of the adult rats are taken from the previous investigation on the intestinal dipeptidases in the adult rat (Josefsson and Lindberg 1966)

It is apparent from the figure that glycyl L-leucine dipeptidase activity is low between the 15 and 18 days *post coitum* In the 18 1/2 day fetuses, however, the activity starts rising to reach a maximum around parturition In animals allowed to suck the activity then abruptly falls already after some hours of life to the same relatively low level as that of the adults The curves obtained for L alanyl L glutamic acid, glycylglycine and glycyl-L-valine dipeptidase activity were similar to that for glycyl-L-leucine However, the results for L-alanyl-L proline dipeptidase activity differ (Fig 3) In this case the activity, which became maximal in those rats taken immediately after delivery, did not fall abruptly in the suckled newborns, but declined successively during the postnatal suckling period, to reach the level of adult rats first about 3 weeks post partum Table 1 gives a survey of the various dipeptidase activities at certain characteristic stages of the development

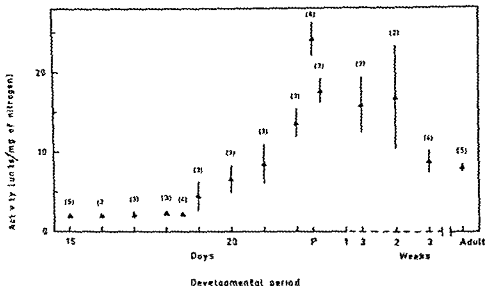


Fig 3 Pre- and postnatal development of L-alanyl L-proline dipeptidase activity (units per mg nitrogen, mean values ± S E M) in the rat small intestine The S E M for 15 days *post coitum* is ± 0 14 for 16 days ± 0 24 for 18 days ± 0 18 and for 18 1/2 days ± 0 15 (bars covered by symbols) Figures in parenthesis indicates number of litters P = partus Optimum pH and 40

TABLE I The pre- and postnatal development of the dipeptidase activities (units per mg nitrogen mean values ± S E M (n)) in the rat small intestine Optimum pH The assays of glycyl glycine dipeptidase activity was determined in the presence of 5 *μmoles* Co^{2+} ions (final concentration 2 9[illegible] 10 [illegible]M)

Developmental stage	L-Alanyl L glutamic acid	L Alanyl L-proline	Glycyl glycine	Glycyl L-leucine	Glycyl L valine
15 days post coitum	3 12 ± 0 64 (4	1 96 ± 0 14 (5)	3 03 [illegible] 1 78 4	6 10 [illegible] 1 [illegible] [illegible]	9 6[illegible] [illegible] 0.66 4
19 days post coitum	10 9 [illegible] 2 49 3)	4 5[illegible] [illegible] 1 76 3	4 19 [illegible] 0 [illegible]8 3	[illegible]6 [illegible] [illegible] 4 [illegible]8 3	[illegible]6 [illegible] [illegible] 3 99 3
[illegible] days post coitum	31 [illegible] ± 7 27 3	13 6 ± 1 [illegible]0 (3	15 8 [illegible] 1 10 3	83 6 [illegible] 20 [illegible] 1	60 [illegible] [illegible]83 [illegible]
Newborn (nonsuckled)	26 9 [illegible] 3 [illegible] 6	24 [illegible] [illegible] [illegible]0[illegible] [illegible]	1[illegible]4 [illegible] 3 [illegible]8 [illegible]	8[illegible]3 [illegible] [illegible]23 [illegible]	8[illegible] [illegible] [illegible]36 [illegible]
Newborn suckled	10 9 [illegible] [illegible]81 3	1[illegible] [illegible] 1 4 3	14 [illegible] 1 8[illegible] 3	[illegible] 9 [illegible] 4 [illegible]	40 [illegible] 4 95 1
3 weeks post partum	11 4 [illegible] 3 73 4	8 90 [illegible] 1 [illegible]1 4	[illegible]86 [illegible] 1 4	[illegible] 1 [illegible]	3[illegible] 0 [illegible] 41
Adult*	10 8 [illegible] 1 [illegible]1 10	[illegible] 3	4 [illegible] 1 1 [illegible]	[illegible] 1	[illegible] [illegible] 24 (10

* From Josefsson and Lindberg 196[illegible]

According to the Students t test the values of the non suckled and suckled newborns differ significantly for the activities of L-alanyl L glutamic acid ($p < 0.05$) glycylglycine ($p < 0.05$), glycyl L-leucine ($p < 0.001$) and glycyl L valine ($p < 0.001$) For L alanyl L proline dipeptidase activity the difference is not significant ($0.1 > p > 0.05$)

The regional distribution of the dipeptidase activities along the small intestine was studied from 19 days *post coitum* The activities for L alanyl L glutamic acid glycylglycine glycyl L leucine and glycyl L valine were in all stages higher in the distal portions of the small intestine as compared to the proximal part These findings were more marked in intestines from the 21 day stage up to and including the newborn period On the other hand L alanyl L proline dipeptidase activity was equally high in the various portions of the intestine in the prenatal period In the following postnatal stages all the activities were slightly higher in the middle and distal sections of the gut thus resembling the conditions found in the adult rat intestine (Josefsson and Lindberg 1966)

Influence of pH and metal ions

The effect of pH and bivalent metal ions on the intestinal dipeptidases was investigated in fetuses aged 19 20 and 21 days *post coitum* Various phosphate and borate buffer solutions were used to obtain a pH range of 6.0 to 8.0 in the digest mixtures The pH optima found for the various activities were as follows (pH optima for adult rat intestinal dipeptidases (Josefsson and Lindberg 1966 are given in parenthesis) for L alanyl L glutamic acid dipeptidase 7.2 (7.3) for L alanyl L proline dipeptidase 7.0 (7.0) for glycylglycine dipeptidase 7.5 (7.5) for glycyl L leucine dipeptidase 7.4 (7.5) and for glycyl L valine dipeptidase 7.5 7.4)

The influence of four bivalent metal ions (Co^{2+} Mn^{2+} Mg^{2+} and Zn^{2+} ions) when added in the concentration of 10 mμmoles (final concentration 5.9×10^{-4} M) on the various dipeptidase activities was essentially the same as that found in the adult rat (Josefsson and Lindberg 1966) However a difference was found for the glycyl L valine dipeptidase activity which was reduced by Co^{2+} ions by about 35 % in the fetuses while these ions were without influence on this activity of the adult rat

Discussion

The present results on the development of the small intestine (jejunum ileum) demonstrate that after a period of cell proliferation in the striated epithelium the mucosa shows a rapid differentiation from the stage of 18 days *post coitum* at parturition 4 days later the villi appear well developed At 18 1/2 days *post coitum* the epithelium in the portion of the small intestine is of a simple columnar type A delicate brush border can be observed at this stage throughout the small intestinal anlage Typical goblet cells are found at 20 days The first sign of crypt formation occurs at 22 days *post coitum* In principle the present findings agree with those

earlier reported (Kammeraad 1942). In the specimens taken 3 weeks after birth both the crypts of Lieberkühn and the villi have essentially reached final development.

From 21 days *post coitum* the epithelial cells, especially in the distal small intestine, contain PAS-positive inclusions, which probably are of muco- or glycoprotein nature (Owman 1963). The inclusions may be associated with the perinatal transmission of immune proteins occurring *via* the gut (Halliday 1955 a, Brambell and Halliday 1956, von Mayersbach 1958, Clark 1959, Owman 1964 b). By this route the rat offspring achieves most of its passive immunity from the mother up to 3 weeks postnatally (Halliday 1955 b and 1956).

When relating the structural development of the mucosa to that of the intestinal dipeptidase activities, certain observations should be pointed out. Thus it is evident that the increase of the enzyme activities (expressed as units per mg nitrogen) from the low level in the cell proliferation period coincides with the differentiating period of the mucosa. It is also apparent that the activities reach maximal values at the end of the latter period. Structural changes in the mucosal cells related to this increase are difficult to establish. The anlage of the crypts of Lieberkühn are not seen until 22 days *post coitum*, at which stage the activities already are maximal or almost maximal. Goblet cells of typical appearance are not present until the 20 days stage. It may be of interest that the brush border membrane appears simultaneously along the whole length of the small intestine at 18 1/2 days, the stage when the enzyme activities begin to increase.

Larger amounts of the activities, except of L-alanyl-L-proline dipeptidase activity, is found in the middle and distal thirds of the intestine. It should be noted that in these portions of the small intestine immune protein is absorbed perinatally (see above), and the first structural sign of this uptake is observed in the form of PAS-positive granules at the 21-day stage.

The newborn period requires special attention. The abrupt fall of the activities, except of L-alanyl-L-proline dipeptidase activity, in the suckled newborns is remarkable. The decrease, which was observed in all the three portions of the intestine, but tended to be more pronounced in the proximal two thirds, coincides with the appearance of increased amount of PAS-positive material both in the intestinal lumen and within the supranuclear cytoplasm in the mucosal cells. This material is probably derived from colostrum, unlike the PAS-positive granules found prenatally, which arise from the absorption of amniotic fluid (Owman 1964 b). Control experiments (Lindberg, unpublished observation) revealed that colostrum obtained from lactating rats has an inhibitory influence on the enzyme activities in intestinal extracts from fetuses aged 22 days as well as from adult rat. This inhibitory effect was less pronounced for L-alanyl-L-proline dipeptidase activity than for the other activities, a finding which may explain [illegible] decrease of this activity in the suckling period. The nature of the inhibitor [illegible] colostrum is under investigation. It should be recalled that the [illegible] species which acquire passive immunity by [illegible] colostrum in the suckling period (Halliday 1955 a). Besides the possible effect of colostrum, other factors [illegible] bacterial invasion and

also hormonal influences, which can affect the enzyme activities in the mucosa in the perinatal period (cf Moog 1959, Ross and Goldschmidt 1955, Halliday 1959), must be considered

The development of the intestinal dipeptidases has previously been investigated in man (Lindberg 1966 a) The study included fetuses between 62 mm and 215 mm of crown rump (C—R) length (corresponding to 11 and 23 weeks of fertilization age) The results from the study demonstrated that the activities were equally high in the smallest fetuses as in the largest Further, the results from an investigation on adult humans (Lindberg 1966 b) showed that the dipeptidase activities in the small intestine were of about the same magnitude in the adults as in the fetuses Thus, in man the dipeptidase activities develop before the fertilization age of 11 weeks When comparing the formation of the small intestinal mucosa of the human fetuses with that of the rat it is found that the structure of the mucosa in the smallest human fetus examined (80 mm C—R) is comparable with that of the new-born rat From the works of Johnson (1910) and Patzelt (1931) it is known that the human mucosa differentiates in fetuses between 18 mm and 55—60 mm C—R (corresponding to a fertilization age of 7 to 10 weeks, Patten 1953) Hence, it is likely that also in man the development of the intestinal dipeptidases is associated with the differentiating period of the mucosa

Miss G A Andersson and Ulla Britt Nilsson gave skilful technical assistance The investigation was supported by grants from Semper Fond för Näringsforskning the Swedish Nutrition Foundation, Albert Påhlssons Fond and the Medical Faculty of the University of Lund

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Acid-Base Changes in Rat Brain Tissue during Acute Respiratory Acidosis and Baseosis

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Received 7 March 1966

Abstract

Pontén, U. *Acid base changes in rat brain tissue during acute respiratory acidosis and baseosis.* Acta physiol. scand. 1966. *68*. 152—163.

The CO_2 buffer capacity of brain tissue *in vivo* was studied by measuring the tissue bicarbonate concentration at various tissue CO_2 tensions in rats which were either hyperventilated or breathed various CO_2 concentrations spontaneously for 30 min. Measurements of the tissue lactate content indicated that the technique of freezing the tissue *in situ* did not lead to tissue hypoxia. The main increase in the tissue bicarbonate concentration at a given inspired CO_2 concentration occurred within 30 min, but there was a slow further increase from 30 to 180 min. The values did not differ significantly between groups anesthetized with pentobarbital or phenobarbital and they were not

An adequate description of the acid base metabolism of a system involves the definition of any change in terms of respiratory and nonrespiratory ('metabolic') components. This has been done systematically for blood, although the best way of defining the respiratory and the nonrespiratory changes has yet to be agreed. Such changes have been derived either from *in vitro* titrations with various CO_2 concentrations, or from changes in the plasma when the organism is titrated with CO_2 *in vivo* (see discussion by Astrup, Siggaard Andersen, Relman and Schwartz in symposium edited by Nahas 1966).

Apart from blood, comparatively little is known about acid base changes in tissues. The brain presents peculiar problems since it has a specialized extracellular fluid, the volume of which is difficult to measure, although it is possibly identical in composition to the cerebrospinal fluid (CSF). *In vitro* equilibrations of brain tissue homogenates with various CO_2 concentrations (Siesjö 1962 b, Kazemi and

Mithoefer 1964) will be of value for certain problems, but the procedure cannot give information on the buffer capacity of the intact tissue because extrapolations from such homogenates are of doubtful validity (Siesjo 1962 b), and the procedure disrupts the cell membranes, thereby abolishing the compartmentation of buffer groups It is likely that many such groups in the intact tissue exist in compartments which have a pH very different from that in the homogenate, and that homogenization will affect the buffer capacity

The present experiments were made to determine the *in vivo* buffer capacity of rat brain tissue The work was made possible by the development of a sensitive and accurate method for the determination of the acid-labile CO_2 of tissues frozen *in situ* with liquid nitrogen (Ponten and Siesjo 1964 a, b), and by the determination of the CO_2 tension gradients in the tissue (Ponten and Siesjo 1966) The buffer capacity of the tissue agreed fairly well with that of whole blood *in vitro* although it was less than that obtained on cat brain tissue by an independent method (Roos 1965)

Methods

Experimental procedures 126 male albino rats of the Sprague Dawley strain weighing 200—400 g were anesthetized with intraperitonal pentobarbital or phenobarbital (60 and 100 mg per kg b w respectively) One femoral artery was cannulated for monitoring the blood pressure and for blood sampling At each sampling the blood loss was 0 1—0 5 ml The total blood loss during each experiments was 1—3 ml which was replaced with a balanced Krebs Henseleit solution (Krebs 1950) The mean hemoglobin content of the blood fell from 15 9 ± 1 6 to 15 2 ± 1 4 g per cent mean ± S D) during the experiment If the blood pressure fell below 80 mm Hg and if this could not be corrected within a few minutes the whole experiment was discarded The animal was laid on a metal box which could be heated at intervals to prevent hypothermia and its head was secured in a head holder At the end of the operation the skin over the skull was incised longitudinally to accommodate a paper funnel for freezing of the brain *in situ* (see below) The body temperature was measured in the rectum using a thermocouple electrothermometer

When the operative procedures were finished the animal was left undisturbed for 10 min after which arterial blood was sampled The animal then breathed air or CO_2 mixtures spontaneously or it was hyperventilated for a set period of 15 min to 3 hrs The gas mixtures contained 0—18 % CO_2 and 30—50 % O_2 The animals were hyperventilated with air or 50 % O_2 using a miniature respiration pump (Palmer London) During these periods one to three arterial samples were drawn the last one immediately before the head of the rat was frozen If the P_{aCO_2} of the two preceding samples differed by more than 10 per cent the experiment was discarded

The technique of freezing the tissue *in situ* has been modified several times Originally Ponten and Siesjo 1964 b) the whole rat was frozen by direct immersion into the liquid nitrogen The preliminary account of the *in vivo* buffer capacity of the rat brain (Ponten 1964 was based on experiments in which only the heads were dipped into the liquid nitrogen In the present experiments liquid nitrogen was poured onto the exposed cranial bones of the vertex through a paper funnel fitted into the skin incision This technique made it possible to freeze the brain without further manipulation and pulmonary ventilation was not obstructed during the freezing Provided the level of anesthesia was adequate there was only a slight stimulation of the respiration during the freezing and spontaneous respiration continued for [illegible] sec after the onset of the freezing The

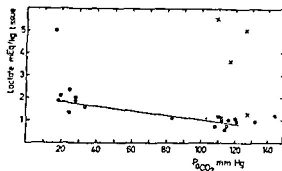


Fig 1 Relation between the P_{aCO_2} and the lactate content of brains frozen *in situ* with different techniques Rats, pentobarbital anaesthesia The regression line refers to brains frozen by pouring liquid nitrogen onto the exposed frontoparietal bones (present method, filled circles). Crosses denote lactate values of brains frozen by immersing the whole head of the rat in liquid nitrogen These brains had low T_{CO_2} values Unfilled circles give the lactate values obtained from brains frozen with the same technique but giving high T_{CO_2} values comparable to those obtained with the present method

joined with a glass stoppered side tube (cf Siesjo and Thompson 1965) Both the main flasks and the side tubes has sockets used for perfusing the unit with CO_2 free nitrogen gas With these modifications the coefficient of variation was still 0 9 per cent The analyses were carried out on the supratentorial parts of the brain and thus the samples included ventricular as well as subarachnoidal cerebrospinal fluid

The water content of the tissue was determined by drying pieces of tissue to constant weight at 105° C

The bicarbonate concentration of the brain tissue (meq/kg) was calculated by subtracting the

derived value was the *mean bicarbonate concentration of the combined water phase of the brain* This parameter was plotted against the mean tissue CO_2 tension to yield a CO_2 binding curve In order to facilitate comparisons the equivalence of the pH term in a tissue system $-\log \frac{(HCO_3)}{P_tCO_2 \cdot S_t}$ — was also calculated In this ratio S_t is equivalent to 0 0314 mmoles/g tissue H_2O/mm Hg (cf Siesjo 1966 Siesjo and Pontén 1966 b)

Results

Influence of the freezing technique on the total carbon dioxide and the lactate content of the tissue

The present study showed a higher tissue buffer capacity than that preliminary reported (Pontén 1964) This was due to a difference in the total CO_2 content of the tissue in hypercapnia, the new series giving the higher values In hypocapnia, however, there was no significant difference in T_{CO_2} between the two sets of experi-

ments In order to study whether the difference observed in hypercapnia was due to the modification of the freezing technique, control experiments were carried out with determination of total CO_2 and of lactate content in tissues from animals which were either exposed to 15 per cent CO_2 or hyperventilated for 30 min

The modified freezing technique used in the present experiments led to consistent values for total CO_2 and lactate content However, if the freezing technique of the previous series (Ponten 1964) was reproduced, both the lactate and the total CO_2 values were variable (Fig 1) Thus, in all but one of the rats frozen with the previous technique and in which special care was taken to protect the airways from the liquid nitrogen, the T_{CO_2} values equalled those observed with the present freezing technique and the lactate content was normal In 3 out of 5 similar experiments which gave low T_{CO_2} values the lactate content of the tissue was increased above the normal The results suggest more than one reason for the observed differences Obviously, the freezing technique previously used could cause a hypoxic condition as indicated by the increase in lactate but it was also found that the CO_2 of the gas mixture breathed by the rat could solidify if the liquid nitrogen made direct contact with the gas tubings In such a case the CO_2 concentration of the inhaled gas mixture could fall to very low values, as verified by passing the gas through a CO_2 electrode

The original freezing technique used in the laboratory (Ponten and Siesjo 1964 b) apparently interfered with the oxygenation of the animal *ante mortem* since it resulted in high tissue lactate values Thus 14 rats anesthetized with pentobarbital were plunged into liquid nitrogen without previous treatment The mean tissue lactate concentration in this group was $4\ 5 \pm 0\ 4$ meq/kg

Comparison between pentobarbital and phenobarbital anesthesia It has been reported (Schmahl 1965) that the redox quotient lactate/puryvate in brain tissue will increase more from the normal value with pentobarbital than with phenobarbital The latter drug is also associated with a higher ATP/ADP, and a higher phosphocreatine/creatine ratio in the tissue Since all these substances should participate in the buffering of CO_2 in the tissue, a comparison was made between two groups of 17 and 40 rats, anesthetized with pentobarbital and phenobarbital, respectively There was a slight difference in the *plasma* bicarbonate but not in the *tissue* bicarbonate concentrations between the two groups, phenobarbital giving a higher mean plasma standard bicarbonate ($24\ 0 \pm 0\ 4$ meq/l, mean $\pm$ SE) than pentobarbital ($22\ 3 \pm 0\ 8$ meq/l) ($p < 0\ 05$) The tissue bicarbonate did not differ by more than 0 3 meq/kg H_2O

Influence of the inspired oxygen concentration This was varied in order to evaluate the possibility of tissue hypoxia during marked hyperventilation ($P_{aCO_2} < 25$ mm Hg) A comparison was made between the tissue CO_2 values measured in a group of 11 animals hyperventilated with air and those measured in another group of 29 animals hyperventilated with 50 per cent O_2 in nitrogen In these and in following groups the individual values were corrected for difference in P_{aCO_2} by means of a parallel displacement of the points along a regression line calculated for the whole groups The reference P_{aCO_2} value chosen was 15 mm Hg The tissue CO_2 values in the two

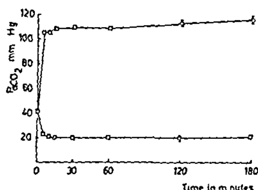


Fig 2

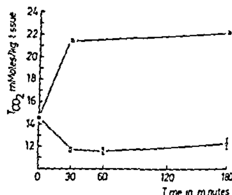


Fig 3

Fig 2 barbit CO_2 in lica tions in the lower curve

Fig 3 Changes in brain total CO_2 content during acute respiratory acidosis and baseosis Rats pentobarbital or phenobarbital anesthesia Exposure to CO_2 concentrations of about 15 % (upper

groups were 11 2 ± 0 2 and 11 0 ± 0 1 meq/kg of tissue H_2O (mean ± SE) respectively The difference is not significant ($p > 0.1$)

The water content of the brain tissue did not show any significant variation with the anesthetic with the O_2 concentration of the inspired gas used or with the P_{aCO} level The mean and SD from the 57 expts used for deriving the CO_2 binding curve was 78 2 ± 0 6 per cent after correction for the difference in water content of the 3 per cent blood in the tissue

Time course of changes in P_{aCO_2} and in the total tissue CO_2 content An acute steady state ' was defined from measurements of the P_{aCO_2} and the tissue CO_2 content at various times after a step change in the ventilatory state (see discussion The step changes consisted of hyperventilation (300 ml per min mean body weight 370 ± 10 g) or exposure to 15 per cent CO_2 The P_{aCO} values were found to stabilize after 10—15 min Fig 2 shows the response of two groups of 5 and 8 animals which were exposed to CO_2 or hyperventilated respectively In hypercapnia a steady P_{aCO_2} level was recorded between 15 and 60 min When the hypercapnia was maintained for 2 or 3 hrs there was an inconsistent further increase in P_{aCO_2} in three animals In hyperventilation at constant volume the P_{aCO} level was constant from 10 to 180 min The same general pattern was found in the intermediate P_{aCO_2} regions

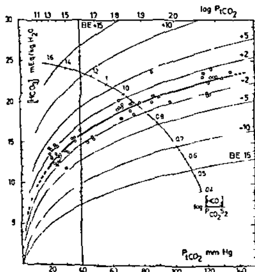


Fig 4

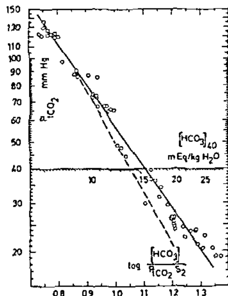


Fig 5

Fig 4 Relation between the mean bicarbonate concentration in brain tissue and the mean tissue CO tens on The

ulation of the buffer capacity (see text)

Fig 5 Relation between log P_{tCO_2} and the logarithm of the buffer ratio (log HCO_3/P_{tCO_2} S_2) The same material as in Fig 4 The solid line is the linear regression line of the present material The broken line denotes the buffer capacity of cat brain tissue as calculated by Roos (1965) from data obtained with the DMO method

The preliminary experiments showed that there were only small changes in the tissue CO_2 content from 30 to 180 min after a change in the ventilatory state (Ponten 1964) Experiments with the present technique verified the observation that the main change in T_{CO_2} occurred within 30 min However, in hypercapnia, there was a slow further change in T_{CO_2} over the next 2 5 hrs, as shown in Fig 3 The additional change seen between 30 and 180 min was about 10 per cent of the entire change

The CO_2 binding curve for brain tissue in vivo On the basis of the above results an "acute steady state" was considered to be reached after 30 min In order to establish the CO_2 buffering of the tissue over a wide P_{CO_2} range 57 expts were carried out at varying P_{aCO_2} values, all rats being exposed to the respiratory change *for 30 min* The results were plotted in a P_{tCO_2} HCO_3 diagram (Fig 4 The regression line thick line in Fig 4) was obtained by fitting a theoretically derived equation to the experimental data (see below) The regression line shows that the tissue bicarbonate concentration at a P_{tCO_2} of 40 mm Hg was 16 0 meq kg of tissue water, and further

that the tissue bicarbonate concentration increases from 12.5 to 22.5 meq/kg H_2O between the P_{tCO_2} limits of 20 to 120 mm Hg. The 95 per cent confidence limits calculated for the whole material were $\pm$ 2.2 meq/kg H_2O.

Theoretical derivation of an equivalent CO_2 buffer curve. Valuable information about the *in vivo* buffer curve can be obtained from a theoretical buffer system. Most conventionally, a one-compartment equivalent buffer system is considered which besides the CO_2 buffer system — $P_{CO_2} \cdot S_t \cdot K'_{H_2CO_3} = (H^+) \cdot (HCO_3^-)$ — contains only one non-bicarbonate buffer system — $(HA) \cdot K_{HA} = (H^+)(A^-)$ — with a total buffer concentration of C mmoles/kg H_2O ($C = A^- + HA$). In a system where Na^+ stands for all cations except H^+, and Cl^- for all nonbuffer anions, the following electrical neutrality equation will hold within 2 per cent for the pH range 5.0—7.8 (Siesjö and Pontén 1966 b).

$$(Na^+) - (Cl^-) = (HCO_3^-) + (A^-) = (BB) \quad (1)$$

In this equation (BB) is the buffer base concentration, i.e. the total buffer anion concentration (Singer and Hastings 1948).

After substitution from the equations given in the text above the following equation is obtained

$$P_{tCO_2} = \frac{K_{HA} \cdot (HCO_3^-)}{K'_{H_2CO_3} \cdot S_t} \left(\frac{C}{(BB) - (HCO_3^-)} - 1 \right) \quad (2)$$

This equation contains 2 variables which can be obtained from the *in vivo* experiments, *viz.* P_{tCO_2} and (HCO_3^-). Moreover, it contains the two constants $K_{H_2CO_3} = 10^{-6.1}$ and $S_t = 0.0314$ mmoles/kg H_2O/mm Hg and the three unknown parameters K_{HA}, C and BB. Derivation of a minimum buffer concentration (see below) requires that the most favourable K_{HA} is used. Since the midpoint of the experimental curve corresponds to a pH of 7.1 in a one-compartment system the most favourable K_{HA} should be $10^{-7.1}$ (maximal buffer capacity occurs when pK_{HA} equals pH, see van Slyke 1922). If this value of K_{HA} is inserted into the equation the remaining parameters C and (BB) would be defined from two points on the curve. Approximate values were obtained by pooling the observations into 5 groups. By inserting this approximate value for one of the parameters the other parameter could be calculated from each experiment. The regression equation obtained from these procedures was

$$P_{tCO_2} = \frac{(HCO_3^-)}{0.314} \left(\frac{35}{36 - (HCO_3^-)} - 1 \right) \quad (3)$$

This equation, which not only fits the experimental points mathematically, but has a physicochemical meaning in that it describes the buffer capacity of a one compartment buffer system, will make it possible to derive the following quantities.

1) *Minimum buffer concentration*, i.e. the buffer concentration needed to account for the buffer capacity of the tissue *in vivo* (Siesjö and Pontén 1966 b). At a K_{HA} of $10^{-7.1}$ the minimum buffer concentration is calculated to 35.1 $\pm$ 0.4 mmoles/kg H_2O. If the buffer acids which are responsible for the buffering of CO_2 have K_{HA} values differing from $10^{-7.1}$ an even higher buffer concentration is needed (see Discussion).

2) *The buffer base concentration (BB)* is calculated to 35 7 ± 0 2 meq/kg H_2O If fixed ' acids or bases are added to the system they will cause a mole for mole change in BB The amounts added will be the

3) *Base excess* values (BE = BB 36) (Astrup *et al* 1960) which express quantitatively the nonrespiratory acid base changes in the system The base excess values have been entered into Fig 4 as thin lines parallel with the buffer line, positive values expressing additions of base and negative ones additions of acid

4) *The standard bicarbonate value*, *i e* bicarbonate concentration referred to a constant CO_2 tension (Astrup *et al* 1960), is obtained for any experimental P_{tCO_2}/HCO_3 pair by displacing the point along the buffer curve to the reference P_{tCO_2} (e g 40 mm Hg) The standard bicarbonate values are then read from the y axis The standard bicarbonate change will express only part of the non respiratory acid base, change, which is equal to BE The ratio between the standard bicarbonate change and the base excess change is 0 7, *i e* an experimentally determined change in standard bicarbonate must be multiplied by 1 4 to give the amount of fixed acid or base which has been added to the system

5) *The buffer capacity* of a system against CO_2 is most conveniently defined as

$$\beta_{CO_2} = d \log P_{tCO_2}/d \log (HCO_3/P_{tCO_2} \; S_t) = d \log P_{tCO_2}/d \log BR \qquad (4)$$

(Siesjo 1962 d), since the relation between log P_{tCO_2} and the logarithm of the buffer ratio (log BR) is a nearly straight line The buffer capacity can be obtained from the diagram of Fig 4 in the following way If two points along the buffer curve are joined with the origin by means of straight lines these lines will cut the log BR scale (circle sector in Fig 4) d log BR is then given by the difference in log BR at the intersection of the log BR scale and the straight lines d log P_{tCO_2} is the horizontal distance between the points on the buffer curve, read at the upper (logarithmic) P_{tCO} scale The buffer capacity thus calculated from the experimental curve is 1 48 and equals the *in vitro* buffer capacity of whole blood containing 12 g hemoglobin/100 ml

Although the buffer capacity can easily be calculated from the HCO_3/P_{tCO_2} diagram it is often visualized as the slope of a linear regression in the log P_{tCO} /log BR diagram Fig 5 shows the present series in this plot The solid line denotes the buffer slope The regression equation was log BR = 2 183—0 674 log P_{tCO_2} The broken line shows the buffer capacity obtained with an independent method Roos 1965) (see Discussion)

Discussion

Experimental techniques Accurate measurements of the total CO_2 content of the brain tissue will require a constant CO_2 tension and an adequate oxygen supply to the tissue during the fixation About 60 sec are required to freeze the central parts of the brain in rats (Everett *et al* 1956 The present freezing technique allowed at least the medullary respiratory and circulatory centers to function for about that time (45—60 sec) which supports the suggestions by Richter and Dawson (1948)

In no instance was the brain frozen *in situ* Moreover, since the CO_2 concentrations of the gas mixtures used were unusually high, ranging from 24 % (Nichols 1958) up to 50 % (Brodie and Woodbury 1958) it is difficult to compare the results with the present data

A systematic study of "intracellular pH" in brain tissue of the cat at physiological CO_2 tensions was recently published by Roos (1965), who calculated his values from distribution of the weak acid 5,5 dimethyloxazolidine-2,4-dione-2 C^{14} (DMO) The two methods, the CO_2 method and the DMO method, should principally give the same information, since both measure the distribution of weak acid in a system The DMO method has the advantage that the tissue need not be frozen *in situ*, but it has the serious disadvantage that the concentration of the unionized form (HDMO) cannot be measured independently or adequately calculated It is possible to calculate the mean capillary or the CSF HDMO concentrations, but because of the phenomenon of nonionic diffusion (Milne, Schribner and Crawford 1958) it is unknown whether the mean intracellular HDMO concentration corresponds to the calculated figures Whereas the mean tissue CO_2 tension is will defined (Pontén and Siesjo 1966), the mean HDMO concentration is not, and the different assumptions used by Roos will give large variations in the bicarbonate concentrations calculated The DMO data of Roos show a buffer capacity of at least 18, *i e* much higher than that calculated from the present CO_2 data This is principally the same difference between the two methods as was obtained by Miller, Tyson and Relman (1963) applying both methods to isolated rat diaphragm (see Siesjo and Ponten 1966 b)

The present work was supported by the Swedish Medical Research Council and by contract No N 62558 3934 between the Office of Naval Research Department of the U S Navy and the University of Lund Technical assistance was given by Mrs Ingrid Glantz Mrs Inga Maj Markinger, Miss Gun Persson Miss Pia Wahlstrom and Miss Lena Rehn

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Acta physiol. scand. 1966. 68. 164—168
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Catecholamine Excretion in Adreno-Demedullated Rats Exposed to Cold after Chronic Guanethidine Treatment

By

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Received 7 March 1966

Abstract

POULIOT, M *Catecholamine excretion in adreno-demedullated rats exposed to cold after chronic guanethidine treatment* Acta physiol scand 1966 68 164—168

Adreno-demedullated rats subjected to a moderate cold exposure showed a significant increase in urinary excretion of noradrenaline (NA) and maintained their thermal equilibrium Adreno-

Exposure to cold is known to produce an important and almost immediate increase in urinary excretion of noradrenaline (NA) (Leblanc and Nadeau 1961, Leduc 1961) Other studies have shown a markedly diminished resistance to cold of rats whose catecholamine stores have been depleted with reserpine (Dandiya, Johnson and Sellers 1960, Zilberstein 1960, Taylor 1961) A closed relationship between cold resistance and urinary excretion of NA was obtained in such treated animals (Johnson 1963) These results and others indicate an important role of NA in the survival of rats subjected to cold However, it is difficult from these studies to determine if the decreased resistance is due mainly to a peripheral or central action of reserpine, since this drug has important depleting effects on both brain catechol and indolamines (Sheppard and Zimmerman 1960) and besides produces a marked sedation

In order to evaluate the importance of the peripheral liberation of catecholamines, we have used guanethidine, a potent sympatholytic drug, which blocks nervous transmission in the noradrenergic postganglionic fibres (Maxwell *et al* 1960) and depletes various peripheral organs of their catecholamine content without affecting amine stores in the brain (Kuntzman *et al* 1962) It is a strongly basic compound

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TABLE I Noradrenaline content of organs after chronic injections of guanethidine (10 mg/kg i. p.) daily for one week Numbers in parentheses indicate the number of animals in each group

	Control (3)	Guanethidine (4)
	μg/kg b w, mean±S E	μg/kg b w, mean±S E
Heart	0 78±0 030	0 11±0 043
Spleen	0 83±0 052	0 092±0 024
Brain	0 40±0 030	0 39±0 013

having such a low lipid solubility that it does not cross the blood brain barrier to an appreciable extent (Kuntzman *et al* 1962) In addition, guanethidine, contrary to reserpine, does not impair the resistance of rats exposed to very low temperatures (Pouliot and Leblanc 1963) In the present work it is attempted to evaluate more precisely the importance of peripheral secretion and action of NA in the defense against cold

Materials and Methods

All experiments were [illegible] 200 [illegible]

into two groups and placed in metabolic cages at 20° C The daily guanethidine treatment was then initiated in one group of animals and the other group served as a control group

The animals were placed in individual metabolism cages coated with plastic to prevent oxidation by metallic ions The cages were mounted on polyethylene funnels at the bottom of which were placed small round bottom flasks placed upside down over a 100 ml beaker to discard the feces. Acetic acid 1 N was added to the urine so that the pH was maintained around 3 Urine collection was started in the morning and continued for the next 24 hrs Each urine specimen was filtered and assayed for free adrenaline (A) and (NA) according to the method of Euler and Lishajko (1961)

In order to determine catecholamines in organs rats were killed by a blow on the head and the organs immediately removed rinsed with cold saline weighed and extracted with trichloracetic acid (10 %) The organs were then assayed for A and NA in essentially the same manner as the urine samples. A Turner fluorimeter model 110 was used for the determinations

Body temperatures were measured by inserting a thermocouple 4—5 cm inside the rectum Guanethidine (Ismelin[R] CIBA) was prepared in an aqueous solution of 10 mg ml and injected intraperitoneally in a dose of 10 mg kg day (expressed as the free base)

Results

Preliminary experiments showed that there is no change in brain catecholamines of rats injected with guanethidine daily for one week whereas there is a 85 to 90 % depletion in heart and spleen (Table I

Adrenal demedullation was followed by a moderate fall in body weight 15—20 g) of the rats Within a week they were back to their initial weight and guanethidine treatment did not cause any further loss in body weight throughout the entire period of treatment The treated rats were not sedated, but showed a mild and transient diarrhea

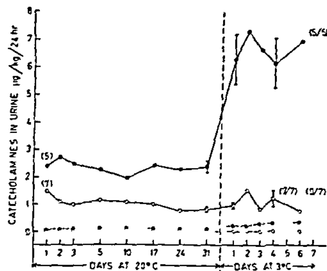


Fig 1 Urinary excretion of catecholamines from adreno-demedullated rats exposed to 20 C and 3° C Numbers in parentheses indicate the number of animals in each group at 20 C and number of rats surviving at 3 C Standard errors are plotted on day 31 at 20 C and days 1 and 4 at 3° C
——— Noradrenaline
— — — adrenaline ● Control rats ○ Guanethidine treated rats

The urinary excretion of A and NA of control and guanethidine treated animals placed first at 20 C and then at 3 C is shown in Fig 1 Urinary NA showed a 50 % decrease in the treated group kept at 20 C P < 0 01) Adrenaline excretion which was low in the control adreno-demedullated rats could not be determined in the treated group Upon exposure to 3 C, the control rats significantly increased their NA excretion P 0 05 within the first 24 hrs and maintained this level for the duration of the experiment Adrenaline excretion was also slightly increased in these animals On the other hand the guanethidine treated group failed to show a significant increase in the urinary excretion of A and NA Six days later the injected rats became hypothermic and died within 48 hrs At this time the control adreno-demedullated rats were still all normal and normothermic

Discussion

The general level of activity is one of the factors regulating body temperature and most drugs with a depressing action on the central nervous system at the same time affect the regulation of the body temperature Euler 1964 It is therefore quite important that a drug should be free of central effects if the correlation between NA secretion from the peripheral tissue stores of catecholamines and resistance to cold stress is to be considered According to Kuntzman *et al* 1962) the action of guanethidine is largely peripheral and does not affect the brain NA content Some authors reported depletion in brain NA after guanethidine Kroneberg and Schumann 1962 Pfeifer, Vizi and Satory 1962) and it is difficult to explain this discrepancy except that there could be strain differences However even in these reports the depletion is quite moderate and no overt behavioural changes are observed In our experiment guanethidine treatment for 7 days had no effect on the NA content in the central nervous system (Table I

As shown in Fig 1, the urinary output of NA is significantly decreased in chronically guanethidine treated adreno-demedullated rats kept at 20° C Moreover, if the same group is exposed subsequently to 3° C, no significant increase in NA excretion was noted and all died 7 days later, only a few hours apart The control animals, in contrast, upon exposure to 3° C showed a threefold increase in urinary NA within the first 24 hrs, which was maintained throughout the duration of the experiment No death occurred in this group and all animals were still normothermic at the end of the experiment Since no change in the NA tissue content was noted in the control the increase in the NA excretion following cold stress is likely to be the result of an increased release, followed by resynthesis The failure of the guanethidine treated rats to increase their NA excretion suggests that guanethidine prevents either the increase in release or the biosynthesis or both, normally induced by cold exposure

Acceleration of catecholamine synthesis following an increased nervous stimulation has been reported in the adrenal medulla (Hökfelt and McLean 1950, Brygdeman, Euler and Hokfelt 1960) It is likely that the same mechanism occurs in nor adrenergic fibres The sustained increase in urinary excretion of NA in cold exposed rats in the presence of an unchanged NA content in tissues (Leduc 1961), strongly suggests increased synthesis of NA as a result of increased nervous activity On the other hand, it is well known that guanethidine blocks the response of postganglionic noradrenergic fibres to nerve stimulation (Maxwell *et al* 1960, Cass and Spriggs 1961, Boura and Green 1962) apparently by a persistent depolarization of the noradrenergic presynaptic terminals (Chang, Costa and Brodie 1965) Our results would then indicate that the sympathetic blockade can be partly responsible for the lack of acceleration of catecholamine liberation The present results also corroborate those of previous investigators in which it has been shown that cold resistance is dependent upon the liberation and action of NA (Hsieh Carlson and Gray 1957, Leduc 1961, Johnson 1963)

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Acta physiol. scand 1966 68 169—177

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The Effect of Various Types of Fat on the Cholesterol Distribution in the Rat

By

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Received 9 March 1966

Abstract

GRAN, F C and R NICOLAYSEN *The effect of various types of fat on the cholesterol distribution in the rat* Acta physiol scand 1966 *68* 169—177

and above the effect of oleic acid The carcass cholesterol of rats given corn oil was slightly lower than in rats given type 2 of fat The difference was statistically significant on cholesterol free diets When various tissues were analysed corn oil induced transfer to other tissues than the liver could not be observed

The fact that polyunsaturated fats reduce the plasma cholesterol level in man and animals is well established likewise that it is a lasting effect The cause of this cholesterol lowering effect is under vigorous discussion, and a number of observations seemingly at variance with each other have been reported In many instances however, with what present day workers (Eneroth Hellstrom and Ryhage 1964 Miettinen Ahrens and Grundy 1965) consider to be inadequate methods extra excretion of bile acids and sterols in feces have been reported following administration of corn oil to hypercholesterolemic man or animal Miettinen *et al* 1965) have extensively reviewed previous work

Most recent work in the field Spritz Ahrens and Grundy 1965 Avigan and Steinberg 1965, Bieberdorf and Wilson 1965) have failed to confirm these earlier reports

It has been suggested by many that cholesterol is transferred from plasma to the tissues following ingestion of corn oil When corn oil has been compared with saturated

fats a reduced plasma cholesterol level with a simultaneous considerable increase in liver cholesterol in the corn oil fed animals has frequently been observed (in rats by Grunbaum *et al* 1957, Avigan and Steinberg 1958, Reiser *et al* 1963, but not by Okey and Lyman 1957, in cebus monkeys by Wissler *et al* 1963) Moore and Williams (1964), on the other hand, found distinctly lower plasma and liver cholesterol in rabbits given a corn oil diet than in rabbits given a saturated fat diet The level of fat in the diet in the above experiments was 15—30 per cent and the experiments lasted from 2 weeks to many months In man Franz and Carey (1961) found that plasma and liver cholesterol was reduced by 9 and 25 per cent, respectively, when 5 healthy men with high normal levels of plasma cholesterol received 3 ozs of corn oil daily for 1 month In a parallel experiment in men given hydrogenated coconut oil the result was a small but insignificant increase in plasma and liver cholesterol

It thus seems to be well established that polyunsaturated fatty acids can induce redistribution of cholesterol from plasma to liver, but it does not seem to be a constant occurrence

In one series of experiments Gerson, Shorland and Adams (1961) have analysed a number of tissues from adult rats given 11 % corn oil in the diet and compared the results with data found in rats given a diet poor in fat They concluded that corn oil transferred cholesterol to various tissues even to the arterial wall

Since their conclusions has attracted wide attention we felt that a repetition and extention of such types of experiments in rats was needed not least in view of the fact that Hauge and Nicolaysen (1959) had found that plasma and liver cholesterol in hypercholesterolemic rats was rapidly reduced by 80 mg soybean oil daily A number of similar experiments has been performed by us confirming and extending the 1959 results (Gran and Nicolaysen 1966)

Experimental

Two series of experiments were conducted

Approximately one year old hooded and albino female rats were taken from the interbred strains of this institute The rats had previously been fed a stock diet containing 7 5 % arachis oil in addition [illegible] fat content of this [illegible]

[illegible] weeks The diet [illegible] rose (by weight) was substituted for fat and cholesterol The rats were then divided into groups and given diets with different combinations of hydrogenated coconut fat corn oil and olive oil as appears from the tables The diet of Hauge and Nicolaysen (1959 was again used but the fat content was increased to 20 per cent at the expense of the equal weight of sucrose One per cent cholesterol was added to the diets when desired

The rats were given their respective diets for the six weeks following The rats were next stunned [illegible]

Analytical Methods

All chemicals were of analytical quality with the exception of the solvents which were of laboratory reagent quality and they were always redistilled before use

TABLE I Comparison of cholesterol methods Liver cholesterol, mg/100 g

Group	Per cent fat in diet	Number of rats	Method: Tschugaeff[1]	Method: Liebermann Burchard[2]	Method: Sperry-Webb[3]
A Coconut fat[4]	19 5	8	550 ± 56	322 ± 20	293 ± 23
Corn oil	0 5				
B Coconut fat[4]	19 5				
Corn oil	0 5	9	3,340 ± 449	3 369 ± 524	3 311 ± 492
Cholesterol	1 0				
C Corn oil	20 0	10	494 ± 39	293 ± 14	252 ± 12
D Corn oil	20 0	8	5 759 ± 255	5 731 ± 255	5 475 ± 227
Cholesterol	1 0				

The values are means ± standard error of the mean
[1] Hauge and Nicolaysen (1958)
[2] Cook (1958)
[3] Sperry and Webb (1950)
[4] Fully hydrogenated

The [illegible]

carcass were weighed out in duplicate for extraction of the lipids

The lipids were extracted with mixtures of chloroform methanol in the proportion 1 2 in the first extraction followed by two extractions where the solvent proportions were 1 1 and 2 1 respectively A solvent volume in ml corresponding to 15—20 times the tissue weight in g was used in each extraction The extracts were heated to boiling on a water bath and thereafter left at room temperature with occasional swirling for the next 30 min The residue was filtered off washed with fresh solvent on the filter, and resuspended in the next solvent mixture

The combined extracts were washed with saline in a separating funnel using a volume of about one fifth of the total solvent volume The solvents were then removed in a rotary evaporator and the residue was taken up in chloroform and diluted if necessary to the desired volume after filtration

The skin was very difficult to homogenize and was therefore left over night in 2 N KOH at room temperature Complete dissolution resulted and the sterols could readily be extracted with ether Recovery studies with added cholesterol indicated that no loss occurred in such a procedure

Cholesterol analyses Serum cholesterol was analysed with the Hauge Nicolaysen method 1958 which uses the Tschugaeff reagents following saponification In work to be published later we have in this laboratory found that the Hauge Nicolaysen and the Sperry Webb procedure yield results which do not differ significantly in serum analyses However when the Tschugaeff principle was applied in analyses of various tissues we found that other substances interfered A systematic study of cholesterol analysis of tissues followed The methods used were 1 Liebermann Burchard reaction directly after saponification as described by Cook 1958 2 the same reaction following precipitation with digitonin (Sperry and Webb [illegible]

TABLE II Comparison of cholesterol methods Carcass cholesterol, mg/100 g

Group		Per cent fat in diet	Number of rats	Method 1	2	3	4
A	Coconut fat[1]	19 5	5	247±21	186±3 0	167±1 5	161±6 4
	Corn oil	0 5					
B	Coconut fat[1]	19 5					
	Corn oil	0 5	5	261±17	223±5 9	184±2 1	188±2 9
	Cholesterol	1 0					
C	Corn oil	20 0	5	285±15	193±9 4	168±3 4	169±7 0
D	Corn oil	20 0	5	312±17	225±10 2	183±5 4	189±9 8
	Cholesterol	1 0					

The values are means ± standard error of the mean

Method 1 Tschugaeff reaction Hauge and Nicolaysen (1958)

2 Liebermann Burchard reaction Cook (1958)

3 Liebermann Burchard reaction after digitonin precipitation Sperry and Webb (1950)

4 Anthrone reaction with digitonid Goodman et al (1963) and Goa (1955)

[1] Fully hydrogenated

Results

Data showing a comparison of methods of cholesterol analysis in various tissues are presented in Table I and II

Liver Only the LBR digitonin method seems to give the true value of cholesterol in livers with normal cholesterol content The direct Liebermann Burchard reaction gave results 10—20 per cent higher, and the Tschugaeff method results which indicate that the reagent used reacts with other material to give erroneously high values However, when the liver cholesterol has increased to several per cent the Tschugaeff method gives an approximately correct value for liver cholesterol

Carcass The difference between the direct LBR and the digitonin LBR was approximately that found with liver With regard to the Tschugaeff reaction the values are again too high The two methods of analysis of the digitonin precipitate showed full agreement between analysis of cholesterol and of the digitonide with anthrone The results exclude the presence of saturated 3-β hydroxysterols which produce no colour in the LBR

For the results recorded in subsequent tables, serum was analysed using the

A and C should first be compared since they represent corn oil versus saturated fat For all samples analysed the values are lower in the corn oil group, however, signifi-

TABLE III Cholesterol in adult rats Skin and carcass mg/100 g

Group	Per cent fat in diet	Number of rats	Skin Sperry Webb	Carcass Liebermann Burchard	Carcass Sperry Webb
A Coconut fat[1] Corn oil	19 5 0 5	12	226±7 3	190±3 4	172[2]±3 0
B Coconut fat[1] Corn oil Cholesterol	19 5 0 5 1 0	11	225±5 4	212±4 3	180 ±3 4
C Corn oil	20 0	12	226±9 4	190±4 3	163[2]±3 2
D Corn oil Cholesterol	20 0 1 0	12	226±11 8	217±7 2	177 ±5 7

The values are means±standard error of the mean
[1] Fully hydrogenated
[2] Group A versus group C $p<0\ 05$

TABLE IV Cholesterol in adult rats Serum and liver

Group	Per cent fat in diet	Number of rats	Serum mg/100 ml Tschugaeff	Liver mg/100 g Liebermann Burchard	Liver mg/100 g Sperry Webb
A Coconut fat[1] Corn oil	19 5 0 5	12	74± 5	319± 23	285± 24
B Coconut fat[1] Corn oil Cholesterol	19 5 0 5 1 0	11	195±18	3 369+524	3 311±492
C Corn oil	20 0	12	67± 4	299± 14	261+ 12
D Corn oil Cholesterol	20 0 1 0	12	292± 6	6 280±349	5 9?2±360

The values are means±standard error of the mean
[1] Fully hydrogenated

TABLE V Cholesterol in adult rats

Group	Per cent fat in diet	Number of rats	Body weight g	Serum cholesterol[1] mg/100 ml
1 Coconut fat	19 5			
Corn oil	0 5	12	245 3 ± 6 9	161 ± 19 5
Cholesterol	1 0			
2 Corn oil	20 0	12	213 8 ± 6 7	262 ± 18 9[2]
Cholesterol	1 0			
3 Coconut fat	12 0			
Olive oil[3]	8 0	12	236 3 ± 6 4	336 ± 22.7[2]
Cholesterol	1 0			

[1] Tschugaeff reaction Hauge and Nicolaysen (1958)
[2] Sperry and Webb (1950)
[3] Skin with hair

cantly so only for carcass cholesterol After addition of cholesterol to the two types of diets considerable hypercholesterolemia developed and the livers contained several per cent of cholesterol A slight increase was observed in carcass cholesterol representing 4 7 per cent in the saturated fat group and 8 per cent in the corn oil group However, the values for the corn oil group do not exceed those for the coconut fat group

The data for the skin free of hair are surprisingly identical for the 4 groups, indicating that cholesterol is not readily accumulated in the skin

The amount of cholesterol accumulated in the liver constitutes several hundred mg, whereas *the group difference for the carcasses are in the range of 10—25 mg per* rat weighing 250 g

The increased liver serum cholesterol ratio in group D compared with group B (21 7 *versus* 17 3) corresponds to results reported by earlier workers The high level of cholesterol in group D we suspected was due to the well known effect of unsaturated fatty acids on cholesterol absorption (Bloomfield 1964, Leveille and Sauberlich 1964)

A second series of experiments was designed to bear on this point As mentioned above, the oils added to the diets in groups 2 and 3 Table V, were added in amounts calculated to ensure that the oleic acid concentrations of the diets were identical. In the preceding series all the material representing various tissues had been exhausted in the work on the *comparison of cholesterol methods* We therefore included in this series also analyses of various tissues Individual analysis was performed with liver and serum only, elsewhere the samples *were pooled and* analysed as one single sample representing all animals in each group

Cholesterol[2], mg/100 g					
Liver	Aorta	Pelt[3]	Muscle	Intestine	Carcass[4]
1 760±170	161	310	70	276	204
6 970±510[5]	157	320	71	302	196
4 900±350[6]	198	310	73	303	210

[4] Carcass minus liver, aorta, skin intestine and muscle sample
[5] Group 2 versus group 3 Serum cholesterol $p<0\ 001$, liver cholesterol $p<0\ 001$
[6] The oleic acid content was equal in the diets of group 2 and group 3

The results obtained with regard to serum and liver cholesterol in groups 1 and 2 are in line with those of the first series The expected effect of oleic acid seems to be obtained The average cholesterol content of the livers in groups 2 and 3 was 746 and 473 mg respectively The results indicate that cholesterol absorption is enhanced by linoleic acid over and above the effect of oleic acid The high liver plasma cholesterol ratio in group 2 compared with group 3 again emphasizes the role of linoleic acid in the redistribution of cholesterol from plasma to the liver

The minor differences observed between other results shown do not appear to warrant too much emphasis Again the carcass cholesterol is lowest in the corn oil group, the data for aorta being inconsistent when seen in relation to plasma cholesterol concentration The higher values for the intestine in the groups given oils may reflect an increased concentration due to increased rate of absorption

The cholesterol values for the skin in this series are distinctly higher in the first series, and this should be so since the skin was analysed with hair in these animals Five samples of hair from normal animals were analysed and the cholesterol content varied from 0 7 to 1 2 per cent We have not studied the problem of cholesterol transfer through the skin to the hair, and we do not therefore know whether the higher cholesterol values of the latter may in part represent transfer of this type However, there is no indication of increased cholesterol concentration due to a corn oil effect

Discussion

The results with regard to cholesterol distribution between plasma and liver in the rats given dietary cholesterol are in line with the results of many previous studies

in which an increased liver/plasma cholesterol ratio was caused by corn oil On the other hand, we could find no indication of a redistribution to other tissues, on the contrary, the carcass cholesterol was significantly lower in the group given 20 per cent corn oil in the diet than in the group given 19 5 per cent coconut fat and 0 5 per cent corn oil Bloomfield (1964) compared the effects of butter and safflower oil (— 20 per cent fat in the diet —) on cholesterol distribution in rats given 0 65 per cent of cholesterol in the diet He found no indication of an increase in cholesterol concentration in other tissues than the liver, results in line with ours When carcass including liver is analysed in rats given cholesterol in the diet, obviously greatly increased values for carcass cholesterol are found (Wilson 1962, Tidwell, McPherson and Burr 1962) On the other hand our results are at variance with those reported by Gerson *et al* (1961) We compared rats given two types of fat in the diet while they compared data from rats given corn oil with those from rats given a diet very poor in fats They report data for free and esterified cholesterol, and the results with regard to 'transfer' of the two cholesterol fractions are inconsistent *e g* in aorta only free cholesterol increased in the pelt only ester cholesterol No data for variability are given in their paper The increase in aorta cholesterol — 39 mg per 100 g tissue — corresponds to 0 034 mg per 80 mg We find the approximate weight of a rat aorta to be 60—80 mg In our analyses of pooled aorta from 12 rats the values for the corn oil group came out as the smallest (Table V) and do not thus support the view of transfer of cholesterol from plasma to aorta mediated by corn oil It has been amply demonstrated that the deposition of cholesterol in the arterial wall occur roughly in proportion to plasma cholesterol in experimental animals Until the same type of results as those reported by Gerson *et al* has been produced in new and extended experiments it seems justified to doubt the biological significance of the type of transfer postulated by these authors Recently Bieberdorf and Wilson (1965) induced hypercholesterolemia in rabbits by feeding them on a diet containing hydrogenated coconut oil When thereafter corn oil was substituted for the saturated fat, plasma cholesterol was reduced as expected, but no effect on synthesis or degradation of cholesterol was found Samples of muscle were collected from the 10 rabbits used, and a nearly constant but statistically not significant increase in muscle cholesterol was found The liver was not analysed however, the contention of these workers being that cholesterol is transferred from plasma to a great muscle mass in a period of declining plasma cholesterol concentration. Such a transfer would imply a change of the membrane of muscle cells so as to increase its content of cholesterol or it would imply an active transport Clearly much better evidence must be produced before such an hypothesis can be accepted As discussed by us (Gran and Nicolaysen 1966) extra sterol excretion induced by corn oil has been found in some experiments, but not in others it then seems reasonable to assume that under such circumstances a redistribution to the liver temporary or permanent has taken place

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Cod Liver Oil Induced Removal of Cholesterol in Hypercholesterolemic Essential Fatty Acid Deficient Rats

By

FREDRIK C GRAN and RAGNAR NICOLAYSEN

Received 9 March 1966

Abstract

GRAN F and R NICOLAYSEN *Cod liver oil induced removal of cholesterol in hypercholesterolemic essential fatty acid deficient rats* Acta physiol scand 1966 *68* 178—183

Rats were given a diet free of polyenoic fatty acids and containing 1 per cent of cholesterol until 150 days old Severe hypercholesterolemia and livers containing several per cent of cholesterol resulted Next 80 mg of cod liver oil was given daily to each rat for 14 days The result was that about 28 mg of cholesterol disappeared from a rat body per day The pathways responsible were not further studied

The effects of various types of fats, included in substantial proportions in the diet, on the distribution and metabolism of cholesterol have been discussed elsewhere (Gran and Nicolaysen 1966) When the level of fat in the diets is 10—40 per cent, numerous mechanisms known to be active in cholesterol metabolism can come into play We refer to a recent discussion by Avigan and Steinberg (1965) and to Gran and Nicolaysen (1966)

In the present work we have used another type of experimental design This followed the pattern common in bioassays, namely the use of animals free of the substance to be tested The dose response curve in such assays is logarithmic The general experience is that the most reliable results are obtained when small doses of the substance to be tested, mostly vitamins, result in a high degree of response In bioassays of vitamin D in rats, we find that doubling the dose from 0 4 to 0 8 units nearly doubles the intensity of cure, whereas an increase to 1—2 units results in only a small extra response Hauge and Nicolaysen (1959) designed a method of this type to study the hypocholesterolemic effects of various types of polyunsaturated fatty acids The present work is a repetition and an extention of the early experiments Since the effect of the polyenoic acids of cod liver oil in depressing serum

TABLE I The effect of 80 mg cod liver oil daily on serum and liver cholesterol in hypercholesterolemic rats free of essential fatty acids

Group	I	II		III		IV	
	Control	Cod liver oil		Control		Control	
Number of rats	5	5		6		5	
Sex	2 m, 3 f	4 m, 1 f		5 m, 1 f		5 f	
		0 day	14 days	0 day	14 days	0 day	14 days
Age, days	146	146	160	212	226	86	100
Body weight, g	128± 8 6[1]	124± 7 5	163± 4 8	133± 4 9	132± 4 4	103± 4 1	107± 6 6
Serum cholesterol mg/100 ml	541± 24	518± 30	356± 34	543± 48	555± 77	386± 18	355± 48
Liver cholesterol mg/100 mg	9,000± 469	8,150± 630[2]	4 530± 598	4 820± 750[2]	4 720± 444	4,090± 598[2]	5,131± 108
Liverfree carcass mg/100 g	321± 21		265± 10				

All analyses by the Tschugaeff reaction (Hauge and Nicolaysen 1958)

[1] The values are means ± standard error of the mean

[2] Liver biopsy

cholesterol was found to be about 4 times that of linoleic acid in vegetable oi (Nicolaysen and Ragaard 1961) cod liver oil was used as the source of polyenoi acids

Experimental

Hooded and albino rats from the ...

... continued on the same diet after weaning and until the experiments were conclude the rats were killed at the ages given in the table

A small blood sample was taken from the tail for the determination of serum cholesterol and th rats to be used were next sele... cholest... to sera... days ... the administration of cod liver oil was started The left half of the median liver lobe was remove while the rats were kept in ether anesthesia the amount of tissue removed corresponded to 1 g of liver) Since ... holesterol concentratio ... re taken

... g blow over the neck ar exsanguinated The methods for tissue analyses have been described by Gran and Nicolays (1956)

Results

The results appear from Table I Serum and liver cholesterol were rapidly reduced in the course of the 2 weeks experiment In the control groups (III and IV) the average values for serum and liver cholesterol remained largely unchanged, indicating that the removal of about 1 g of liver does not affect the cholesterol concentration of the liver in the course of 14 days

The difference in cholesterol concentration in plasma and liver between the 3 groups need some comments The animals used in experiments of this type are selected from a much larger group of rats in which serum cholesterol is analysed the day before the actual experiment is started The selection aims at groups with the same average serum cholesterol level In group II the serum cholesterol level was initially the same as in group III, the liver cholesterol, however, was nearly twice that in the experimental group We have in the course of years found that variation within litters and between litters is considerable, and it appears from Table I that serum and liver cholesterol do not rise in parallel In the earlier experiments (*Hauge and Nicolaysen 1959*) *serum cholesterol* was *425 mg/100 ml* and *liver* cholesterol 3 4 per cent on zero day Following two weeks' dosing of 80 mg soybean oil daily, the values were reduced to 191 mg/100 ml and 1 92 per cent respectively

Thus, group averages vary from time to time in rats given these hypercholesterolemic diets However, this does not weaken the conclusion that cod liver oil induced a reduction of serum and liver cholesterol

The cholesterol concentrations of various tissues and in the carcass were determined, only the values for carcass cholesterol being reported The reason is the following The experiments presented here were done before the experiments in adult rats (Gran and Nicolaysen 1966) were started The erroneously high tissue cholesterol values obtained with the Tschugaeff method have been documented by us Somewhat lower values were found for the cholesterol concentration in tissues analysed in the cod liver oil group than were found in the control group The livers weighed on an average 11 g in the cod liver oil group, and thus contained about 0 9 g of cholesterol of which 0 4 g was removed in the course of 14 days The rats minus liver weighed about 150 g at the end of the experiment A transfer of the cholesterol from the liver to the carcass would imply an increase of cholesterol from 265 to 530 mg per 100 g carcass In consequence we feel justified in concluding that in the course of the experimental period cholesterol removed from the liver was also removed from *the rat Our negative* findings (Gran and Nicolaysen 1966) with regard to transfer of cholesterol to other tissues than the liver lend support to this conclusion

Discussion

The cod liver oil used contained about 25 mg of polyenoic fatty acids chiefly of the penta- and hexaenoic type Under the present experimental conditions a mean disappearance of about 28 mg of cholesterol daily from a rat body was observed The effect of fats rich in polyunsaturated fatty acids on the distribution of cholesterol

in rats and other species when the diets contain 10—30 per cent of such fats has been discussed elsewhere (Gran and Nicolaysen 1966) In the recent years a number of studies on the metabolism of cholesterol under the influence of diets relatively rich in polyunsaturated fatty acids have been published and a brief discussion of the results is needed

In their work on rats Avigan and Steinberg (1958) found evidence of increased synthesis of cholesterol in the liver under the influence of corn oil On the assumption that steady state prevailed in their rats, the data also indicated increased excretion or breakdown of cholesterol Lewis, Pilkington and Hodd (1961) injected plasma lipoproteins containing ^{14}C labelled cholesterol from donor rats into other rats Bile acids and sterol excretion by the recipient rats increased when an unsaturated fat was given to the donor rats

Eneroth, Hellestrom and Ryhage (1964) studied the excretion of fecal sterols in 7 men fed a standardized diet supplemented with butter fat or corn oil Only in 3 of the 7 men were feces collected continuously when they were switched from the butter (14—18 days collection) to the corn oil period (25—32 days collection) The extra excretion of cholesterol and cholesterol neutral metabolites per day in the corn oil period was 63, 122 and 172 mg per day, respectively, in 3 men The cholesterol intake in the butter period was 250 mg, in the corn oil period 130 mg daily, According to Spritz, Grundy and Ahrens (1963) true absorption of cholesterol in man is 62—86 per cent on a cholesterol free diet When dilution of endogenous cholesterol with exogenous cholesterol occurs, true absorption will be reduced and, assuming that 70 per cent of the 120 mg cholesterol ingested in excess in the butter period was absorbed, 36 mg must be added to the above figures for extra excretion of cholesterol in the corn oil period It must be concluded therefore that corn oil caused a highly significant extra excretion of cholesterol in the 3 men In the other collection did not start until 14—16 days after the beginning of the corn oil period the collection periods were correspondingly shorter and more irregular results obtained

In the recent studies by Spritz Ahrens and Grundy 1965 and by Av Steinberg (1965) in a total of 12 men a significant extra excretion of sterols in 2 only

The variability and uncertainties inherent in balance studies are well kn son and Sjogren (1964 1965) have recently made important contributi cussion of this subject In this laboratory we have over decades gained experience from our studies of calcium metabolism in man and animal discussion may be useful When feces are collected say over 2 w sampling error is not zero Markers are needed to reduce the marker e g TiO_2 should be used in one period and another, in the other when results from 2—3 weeks experiments are co term calcium balance study by Malm 1958 in man the fe feces collected over 2 weeks could vary several hundred mg f to the next, the calcium content of the diet being consta

variations occurred lasting for several 2 week periods in which positive balances alternated with negative ones due to the variability in fecal calcium Similar results have been reported for calcium by Ackerman and Toro (1953) and for magnesium by Seelig (1964) Over 40—50 days were reported negative balances of such a magnitude that, were they representative of the calcium and magnesium balance of the persons studied, all calcium and magnesium would have been lost from their bodies in the course of 1—2 years The reason for such results is not clear In studies of digestive juice calcium in rats Gran (1960) found a variability of about 100 per cent occurring in rats from week to week when the food intake was constant

Although much remains to be elucidated, the fact is that significant extra sterol excretion under the influence of corn oil has been found in 5 out of 15 men A *temporary redistribution of cholesterol from plasma to the liver can explain negative* findings Provided synthesis, degradation and excretion do not change when a subject is transferred from a saturated to an unsaturated fat diet such a redistribution may conceivably become permanent It remains a matter of opinion whether the present finding should be taken as evidence of a catabolic (excretion and/or degradation) effect of polyunsaturated fatty acids in cholesterol metabolism in general, irrespective of the amount of fatty acids given Conceivably only small amounts of polyenoic fatty acids may under certain circumstances be required for a normal (maximal?) sterol excretion and/or degradation When oils are given in quantities of 10—30 per cent in the diet the interplay between effects of unsaturated fatty acids on absorption and reabsorption, on synthesis degradation and redistribution between plasma and liver may decide the net result in a given experiment

More detailed studies in man and animals are needed to establish the true picture of sterol balances when the type of dietary fat is altered We concur with the view of *Avigan and Steinberg (1965) that there is at least no necessary* and consistent relationship between fat induced changes in serum cholesterol levels and rates of fecal sterol and bile acid excretion A conclusion to the effect that corn oil does not increase fecal sterol excretion, and therefore transfer cholesterol to the tissues is not justified in view of present day pooled observations

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Acta physiol. scand. 1966. 68 184—192

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Studies on the Ganglionic Site of Action of Sympathetic Outflow to the Stomach

By

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Received 15 March 1966

Abstract

Jansson, G. and J. Martinson, *Studies on the ganglionic site of action of sympathetic outflow to the stomach.* Acta physiol. scand. 1966. 68. 184—192.

In acute experiments on cats anesthetized with chloralose inhibition of excitatory vagal responses of gastric smooth muscle was produced by reflex or direct activation of an adrenergic nerve fibre system. The reflex that could be elicited by distension of a jejunal loop or stimulation of mesenteric afferents was dependent on intact spinal connections because it was blocked by spinal anesthesia. Activation of the adrenergic nerve fibre system did not inhibit gastric myogenic tone or gastric muscle contraction elicited by the muscarinic action of acetylcholine. It was therefore concluded that the adrenergic outflow responsible for this type of inhibition acts on parasympathetic intramural ganglion cells involved in the excitatory control of gastric motility.

According to Bayliss and Starling (1899), the intestines are normally under the reciprocal control of vagal excitatory and splanchnic inhibitory fibres. This postulated arrangement has been generally assumed to hold also for the stomach though no recent studies on this organ seem to have been published (for details regarding earlier work see McSwiney 1931). But observations by Celander (1959) and Kock (1959) on intestinal motility suggest that the inhibitory influence of a supraspinal reflex increase in sympathetic activity is not caused by specific inhibitory fibres but by a reflex release of catecholamines from the adrenal medulla. These observations were difficult to bring into line with the view that specific inhibitory fibres act directly upon the intestinal smooth muscle cells.

The so-called intestino-intestinal inhibitory reflex (Kuntz and Saccomanno 1944, Kock 1959), on the other hand, fulfils most criteria of a direct nervous mechanism, in that it is prompt in onset and can easily be elicited even when the adrenal glands have been removed. Johansson and Langston (1964) showed that it is a true propriospinal reflex, easily evoked in the acute spinal preparation and normally exposed to a more or less dominant tonic inhibitory influence of supraspinal origin (Johansson, Jonsson and Ljung 1965). This arrangement differs from the sympathetic cardiovascular control, where the spinal "centres" are normally facilitated by a tonic supraspinal excitatory influence.

Experimental evidence of a ganglionic action of sympathetic nerves has been published Recently Norberg (1964), who used the fluorescent microscopy technique, demonstrated the existence of adrenergic synapses in the parasympathetic, intramural ganglia of the intestinal wall Kewenter (1965), who used a physiological technique, obtained results suggesting a similar arrangement

As for the gastric smooth muscle, the vagus nerves can elicit two different responses, one group of efferent vagal fibres produces increased motility, another group elicits a profound relaxation of the corpus and fundus (Jansson and Martinson 1965) The latter group of vagal fibres, which are not adrenergic, elicits a far more powerful relaxation than the sympathetic fibres, at least when they exert their influence on the basal activity in the stomach (Martinson 1965) The present investigation was undertaken in order to study the interaction between the efferent vagal fibres and the sympathetic fibres with regard to their effects on the gastric smooth muscle and with regard to the possible ganglionic site of action of the sympathetic outflow, as suggested for the intestines (Norberg 1964, Kewenter 1965)

Material and Methods

Experiments were performed on 42 cats weighing between 1 6 and 4 4 kg The animals were deprived of food for 24—36 hours before use They were anesthetized by i v injection of chloralose (70 mg/kg b w) after a brief induction narcosis with ether Free air passage was secured by inserting a tracheal cannula

The abdominal [illegible]
car [illegible]
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suc [illegible]
lev [illegible]
hy [illegible]
The vagus nerves [illegible]
ends [illegible]
dissec [illegible]
Th [illegible]
jejun [illegible]
The r [illegible]
to a [illegible] bore plastic catheter connected to a vertical tube which served as a water manometer to measure the pressure that could be applied to the jejunal loop with a syringe filled with isotonic saline In 15 expts the nerve bundles along the superior mesenteric artery were cut and electrodes for stimulation were placed on their central ends The left splanchnic nerves were used for sympathetic stimulation

The peripheral ends of nerves to be stimulated were placed in ring-shaped bipolar silver electrodes. The poles were embedded in a perspex material insulating the outside of the electrodes from surrounding tissues The electrodes were connected to a square wave pulse generator Grass model S4 Stimulator) Pulse intensity duration and frequency could be adjusted within wide limits

In some animals the common carotid arteries were dissected free and arranged so as to be easily clamped In these animals the brachial plexus on one side was also prepared for afferent stimulation in the same way as the other nerves

In 10 expts. acetylcholine (1—5 µg kg was injected into the celiac artery by means of a thin polyethylene catheter introduced into the splen[illegible] artery in retrograde direction

Injections of guanethidine (Ismelin® CIBA and atropine sulphate were given i v In some experiments spinal anesthesia was given at the lower thoracic level A thin polyethylene catheter was then inserted through a lumbar laminectomy for subdural administration 1—1 5 ml of 2 % lidocain solution (Xylocain® Astra) was usually sufficient to secure adequate blocking Usually a large volume of dextrane Tyrode solution 10 30 ml had to be given to control the otherwise extensive fall in blood pressure following spinal anesthesia of this type

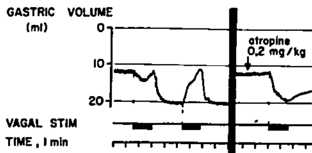


Fig 1 Cat 3 2 kg General appearance of gastric motor responses to vagal stimulation as recorded with volumetric technique Ventral vagus nerve trunk stimulated below the diaphragm 8 imp/sec 2 msec 5 volts Note that atropine had no effect on basal tone

Results

Recordings of the gastric volume at constant, low intraluminal pressure in the chloralose anesthetized and vagotomized cat showed characteristic features (Fig 1) In most cases the volume was initially small and the kymograph record was smooth with rhythmic respiratory movements of almost constant amplitude By administration of atropine (0 1—1 mg/kg) it was shown that the evidently considerable gastric tone was only slightly or not at all dependent on the parasympathetic excitatory nervous influence (Fig 1 right) This gastric tone is therefore hereafter called the basal myogenic tone Application of a vagal stimulation, strong enough to activate both the excitatory and the high threshold 'relaxatory" vagal fibres, produced characteristic responses (cf Jansson and Martinson 1965) When the initial volume was small, the immediate gastric effect was a relaxation (*i e*, an increase of volume), but when the volume was initially fairly large (low initial tone), it produced an initial contraction (Fig 1 left) During stimulation irregular waves of activity in the stomach wall were superimposed on the rhythmic respiratory movements visible in the resting state Upon cessation of the stimulation the stomach rapidly relaxed and when the initial volume was small, this after relaxation considerably increased the volume of the stomach which afterwards slowly recovered pre stimulation size When atropine had blocked the excitatory vagal fibres, the stomach relaxed immediately upon vagal stimulation, provided that the voltage was high enough to activate also the vagal relaxatory fibres (Fig 1 right)

Stimulation of the splanchnic nerves caused gastric relaxation that was quite small and slow in onset, not being considerable until 'supraphysiological' rates of stimulation were applied (Martinson 1965) Moreover, low rates of splanchnic stimulation sometimes produced excitatory effects (Fig 2) The relaxations produced by splanchnic stimulation were essentially the same in magnitude whether the basal 'myogenic" tone of the stomach was initially high or low But when the splanchnic stimulation was performed during continuous stimulation of the vagal excitatory fibres, the inhibition produced by the direct activation of the symphathetic fibres sometimes differed in character Fig 2 illustrates such an experiment Without vagal stimulation graded splanchnic stimulations induced slight excitatory responses at 1 and 4 imp/sec and small and slow inhibitions at higher frequencies With con

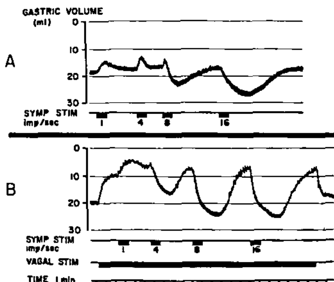


Fig 2 Cat 1 9 kg Effect of stimulation of the left major splanchnic nerve (5 msec 5 volts) on gastric motility before (A) and during (B) vagal stimulation 8 imp/sec 3 msec 5 volts Note the more rapid and potent inhibitory responses to splanchnic stimulation in B Note also the reversal of response to 4 imp sec in B

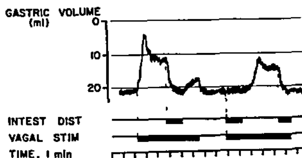


Fig 3 Cat 1 9 kg Low basal tone after preceding vagal stimulation Clear cut excitatory responses are elicited by vagal stimulation (1 imp/sec 2 msec 5 volts) and these responses are effectively inhibited by intestinal distension To the right concomitant intestinal distension completely prohibits the vagal response

tinuous vagal stimulation graded splanchnic stimulation elicited prompt and considerable gastric relaxation with the exception of stimulation at 1 imp/sec These inhibitions induced by direct sympathetic stimulation were so extensive as to suggest that the sympathetic fibres had suppressed the superimposed smooth muscle tone created by the vagal excitatory fibres The inhibitions were however not obtained so regularly as the inhibitory reflex responses described below

Distension of an intestinal loop is known to produce a rapid reflex inhibition intestinal motility (cf Johansson *et al* 1965) But a similar intestinal distension procedure had no influence on the basal myogenic tone of the stomach (Fig 4 and However, intestinal distension during continuous activation of the vagal exciting

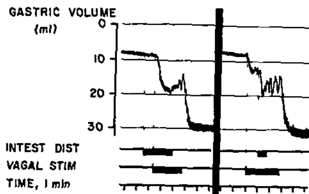


Fig 4 Cat 4 4 kg Appearance of *gastric volume response to* vagal stimulation and intestinal *distension (80 cm H_2O) elicited* from a high basal tone level Note that intestinal distension had no effect on gastric tone when vagus is not stimulated Vagal stimulation 4 imp/sec 2 msec 5 volts

Fig 5 Cat 3 5 kg Comparison between *the effect of* intestinal distension (80 cm H_2O) upon *the contraction of the stomach* elicited by intra arterial infusion of *acetylcholine and by* vagal stimulation (1 imp/sec 2 msec, 5 volts) respectively

fibres regularly inhibited this vagal excitatory influence promptly and often completely (Fig 3 and 5) When the basal tone of the stomach had been lowered by a preceding stimulation of the vagal relaxatory fibres, clear-cut motor response could be elicited upon activation of the vagal excitatory fibres This type of vagal response could be reflexly inhibited by subsequent intestinal distension (Fig 3) and it could be completely prohibited by concomitant intestinal distension (Fig 3 right) When the basal myogenic tone of the stomach was initially high *i e*, when the stomach contained a small volume (Fig 4) the appearance of the vagal and reflex responses was different Intestinal distension had no effect on basal tone (left hand panel) but the gastric relaxation on vagal stimulation during continuous distension (left panel) was more marked than it was when no distension was applied (right panel) Rapid inhibition could, however, be produced by intestinal distension during the vagal stimulation also in this case (right hand panel) The same type of

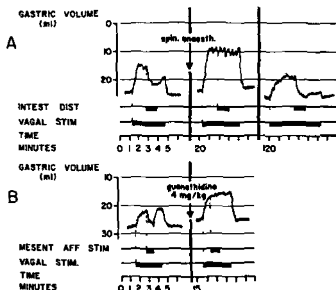


Fig 6 Effects of spinal anesthesia and antiadrenergic drug (guanethidine) respectively on the intestino-gastric inhibitory reflex

A Cat 2 6 kg Cervical vagal stimulation 2 imp sec 2 msec 5 volts intestinal distension 50 cm H_2O Note increased response to vagal stimulation during spinal anesthesia and recovery of response

B Cat 2 3 kg Intra abdominal vagal stimulation 4 imp/sec 2 msec 5 volts Mesenteric afferent stimulation 10 imp/sec 4 msec 3 volts

sympathetically mediated, spinal inhibitory reflex could also be elicited by electrical stimulation of afferent mesenteric nerve fibres (see e.g Fig 7) and exerted the same influence on the vagal effects on the stomach

On the other hand, as in Kock's (1959) studies on the intestine, inhibition could not be significantly induced by reflexly increased sympathetic discharge during carotid occlusion or during high voltage stimulation of afferent fibres in the brachial plexus The sympatho inhibitory fibres to the gastrointestinal tract are possibly not involved in this type of reflex sympathetic activation

The fact that intestinal distension had no influence on the basal myogenic tone of the stomach but promptly inhibited vagal excitatory activity suggested a ganglionic site of action of the sympatho inhibitory nerve endings It was assumed that a non nervous activation of the gastric smooth muscle cells would not be inhibited by the intestino-gastric inhibitory reflex or by direct splanchnic stimulation and it could be shown that the excitatory effect of intra arterial infusion of acetylcholine on gastric smooth muscle was not abolished by activation of the intestino gastric inhibitory reflex (Fig 5)

Spinal anesthesia abolished the inhibitory reflex which recovered as the anesthesia wore off (Fig 6A) — The adrenergic character of the intestino gastric inhibitory reflex was ascertained by studying the response after injection of guanethidine i v.

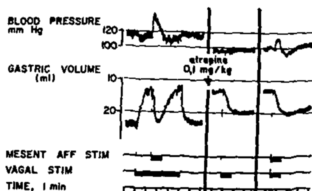


Fig 7 Cat 3 2 kg Left panel Inhibition of vagal cervical stimulation (8 imp sec 9 msec 5 volts) by stimulation of mesenteric afferents (8 imp sec 5 msec 5 volts) Atropine 0 1 mg/kg was given between left and middle panels to reveal vagal relaxation response Right panel Vagally elicited relaxation was unchanged by activation of mesenteric afferents as compared with the middle panel of response Note that blood pressure response to afferent mesenteric nerve stimulation is unaffected

A dose of 1—4 mg/kg regularly abolished the intestino gastric reflex inhibition (Fig 6B) The vagal excitatory responses generally augmented during spinal anesthesia and during adrenergic blocking by guanethidine (see Fig 6) suggesting that a sympathetic influence might have been slightly active throughout

Finally, the action of the intestino gastric inhibitory reflex upon the effects induced by the vagal relaxatory fibres on gastric smooth muscles was also studied Fig 7 shows an experiment of this type After atropine, which blocked the vagal excitatory fibres and produced a pure gastric relaxation upon vagal stimulation the effects of intestinal distension of afferent mesenteric nerve stimulation was studied before and during vagal stimulation There was now no sign of any sympathetically induced inhibition of the vagal relaxation response

Discussion

The present results suggest that an adrenergic outflow to the stomach can effectively inhibit vagally elicited excitatory motor responses to the stomach Procedures such as distension of the intestine or afferent mesenteric nerve stimulation will most effectively activate the sympatho inhibitory fibres responsible for this adrenergic outflow The reflex is considered not to be equivalent to the 'enterogastric' reflex (cf Thomas 1957) as it is elicited from parts of the intestine other than the duodenum and furthermore seems to resemble the intestino intestinal inhibitory reflex of Kuntz and Saccomanno (1944) The elicited reflex might then be termed an 'intestino gastric inhibitory reflex', and the fact that it was blocked by guanethidine proves that it operates by an adrenergic mechanism Furthermore it could be shown that the reflex is dependent on intact spinal connections and that it is not mediated simply via autonomic ganglia, because it is completely abolished by spinal anesthesia This is in accordance with the findings of Johansson and Langston (1964 concerning the propriospinal intestino intestinal inhibitory reflex

The intestino gastric inhibitory reflex cannot however cause relaxation of the gastric smooth muscle cells when their tone is entirely myogenic in origin The often

considerable basal tone in the stomach preparation, as studied in the present experiments, is evidently myogenic in origin, because it is usually not significantly depressed by atropine, whether it is initially high or low (cf Fig 1 and 7) Only the motor effects induced by the vagal excitatory fibres can be effectively suppressed by the intestino-gastric inhibitory reflex Intra arterially infused acetylcholine, acting mainly by its muscarinic effect on the smooth muscle cells directly, causes excitatory gastric responses that cannot be inhibited by the reflex The present results therefore suggest that the influence of the vagal excitatory fibres is blocked by the intestino gastric inhibitory reflex proximal to the neuromuscular junction Kewenter (1965 arrived at the same conclusion regarding the intestine Morphological data have proved the existence of adrenergic nerve terminals around ganglion cells of the myenteric and submucous plexa of the intestine (Norberg 1964) The present observations in the stomach agree with an assumption of this type of arrangement and argue against the occurrence of reflex sympathetic inhibition with an action directly on the myogenic activity of the gastric smooth muscles The smooth muscle cells are evidently only under the direct influence of the high threshold relaxatory vagal fibres distributed only to the corpus fundus region

Several studies on the central nervous influence on gastric motility have dealt with the problems of gastric motor inhibition (for ref see Eliasson 1960) Since, according to the present study, an underlying activity of the vagal excitatory fibres is a prerequisite for showing the inhibition exerted by the adrenergic nervous outflow, its inhibitory influence can seemingly disappear upon section of the vagus nerves, if such division eliminates a centrally induced vagal tone If the difference between such a neurogenic tone, mediated via the vagal excitatory fibres, and a purely myogenic tone is not properly considered it might be erroneously concluded from such experiments that centrally induced inhibitory responses are mediated via the vagus nerves, when they are in fact due to sympathetic inhibition of a prevailing activity in vagal excitatory fibres This might be the case especially in the stomach which in the acutely denervated preparation seems devoid of spontaneous activity in the intramural nerve plexa as discussed above but can nevertheless show a wide variation of myogenic tone

One would expect efferent splanchnic nerve stimulations to elicit similar inhibition of vagal tone as does the activation of the intestino-gastric inhibitory reflex Such inhibitions by direct sympathetic stimulation could undoubtedly sometimes be produced, but not in all experiments suggesting that the splanchnic nerves may contain fibres that interfere with the inhibitory adrenergic system which can be so promptly and regularly activated in the intestino gastric reflex

As pointed out by Kock (1959 with respect to the intestino intestinal inhibitory reflex its efferent link is not activated in connection with the fairly generalized reflex sympathetic excitations produced by *e g* carotid occlusion bleeding or by high voltage afferent somatic nerve stimulation Conversely it can be concluded that a physiologically elicited reflex vasoconstriction of this type cannot *per se* affect myogenic tone in the stomach or the ganglionic neuromuscular transmission

of the vagal control of the stomach Such interferences require blood borne catecholamines

Under the present experimental conditions the intestino gastric inhibitory reflex system obviously showed some tonic activity, since the vagal responses were augmented by spinal anesthesia and by administration of adrenolytic drugs Such a tonic reflex activity might be due to activation of peritoneal pain fibres caused by the abdominal surgery This gives rise to the question whether the reflex system studied might be of physiological significance The possibility remains that it is a purely pathophysiological event dependent only on pain fibre activation It can, however, be elicited rapidly and potently also by rather low intraluminal pressures in the intestine — 20 to 80 cm H_2O — which need not to be noxious stimuli Although the mean pressure within the gut normally is much lower, muscular contractions are able to increase intestinal wall tension considerably when producing so called "type II waves" (see e g Davenport 1966, p 44) The reflex may therefore be involved in some feedback mechanism regulating gastrointestinal motor function (presumably its pressure gradient) It is interesting in this respect to note that the vagal relaxatory nerve fibre system to the stomach is not influenced by the intestino gastric inhibitory reflex system This also fits in with the assumption that the intestino-gastric inhibitory reflex may be specifically arranged and not merely constitute a diffuse blocking of vagal activity

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Responses to Linearly Rising Currents in Frog Skeletal Muscle Fibers

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Received 21 March 1966

Abstract

Knutsson, E. and C. R. Skoglund. *Responses to linearly rising currents in frog skeletal muscle fibers.* Acta physiol. scand. 1966. 68. 193—205.

[illegible] fibers to linearly rising [illegible] latencies up to 1 [illegible]

fibers were correlated to the slow active subthreshold processes occurring [illegible] stimulation with linearly rising currents.

Subthreshold stimulation of skeletal muscle fiber by currents of more than one third of threshold strength gives rise to an active subthreshold response which becomes apparent as a larger depolarization than is to be expected from the passive charging of the cable network of the fiber (Kuffler 1942, Katz 1948) and is also revealed by a conductance change in the membrane (Katz 1942). In a recent investigation (Knutsson 1964) the active and passive subthreshold processes during application of linearly rising currents were extensively studied in frog sartorius muscle and the activation processes were found to vary with the stimulus gradient in a regular manner. Among the results reported were the findings that the active subthreshold potential started at a higher membrane potential value the lower the gradient and that its amplitude increased as the current gradient was lowered.

In a preliminary communication (Knutsson and Skoglund 1963) an account was given of some results obtained in studies of suprathreshold stimulation of frog muscle fibers with currents of different gradients and the present paper will present results from a more comprehensive investigation on spike generation during stimulation with slowly rising currents.

The investigation includes systematic studies of stimulus threshold, firing level and spike configuration during stimulation over a wide range of gradients, and it will be shown that the response patterns of cells with normal resting potential are characterized by a constant firing level and an all or none type of spike response, whereas even a small reduction of the resting potential is associated with a characteristic change into a graded type of response Attempts were made to relate the different types of response pattern to the preceding subthreshold processes

Methods

were performed at room temperature (20—22° C)

Two microelectrodes (tip diametres less than 1 μ resistance 10—20 MΩ tip junction potential about 5 mV) were inserted 30—50 μ apart into an end plate free zone of an individual muscle fiber One electrode was used for stimulation with pulses of linearly rising current from a generator delivering "triangular pulses of varying rate of rise and duration The output of the generator was connected to the microelectrode via a series-coupled capacitor 0 05 μF and a resistor 100 MΩ For measuring the current through the stimulating electrode the voltage drop across a 10 000 Ω resistor in the stimulus circuit was recorded on one of the beams of a double beam oscilloscope after amplification by a d c amplifier In some experiments a constant current delivered from a separate stimulator but applied to the same microelectrode was used to pre set the membrane potential above or below the resting level The other microelectrode was used for recording the membrane potential on the other beam of the oscilloscope via a cathode follower with a capacitance neutralizing feedback (Haapanen and Ottoson 1954) Since the propagated response is set up in the immediate vicinity of the stimulating electrode in this type of stimulation the recorded membrane potential changes correspond closely to the actual potential changes at the point of generation, the inaccuracy introduced by electrotonic spread of slow potential changes between the site of stimulation and the recording point having been estimated to be below 1 mV (Knutsson 1964

Results

Responses of fibers with normal resting potential

Out of the 300 muscle fibers studied about 95 % had resting potentials of a mean value of —88 mV (range —86 to —90 mV) and their responses to linearly rising currents showed close conformity The remaining 5 % of the fibers exhibited exceptionally low resting potentials and more varying response patterns as will be illustrated below

On application of linearly rising stimuli muscle fibers are exposed to current fields of comparatively long duration that may give rise to irreversible membrane changes, and at an early stage of our experiments we found that repeated stimulation might reduce the spike amplitude To minimize such effects the analyses were based on the first four to eight responses in each fiber

Figure 1 shows a typical series of responses to linearly rising currents of successively lowered gradients corresponding to spike latencies of up to about 60 msec within this latency range the spike potentials take off at about the same membrane potential level, are of similar shape and only moderately reduced in amplitude with lowered gradients The strength of the stimulating current at the moment of spike initiation has been marked by gaps in the current recordings which show how the threshold

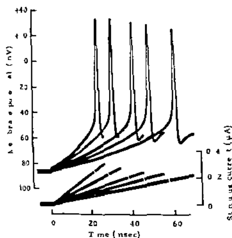


Fig 1

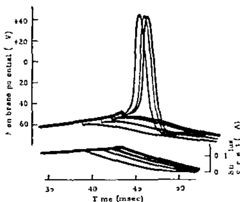


Fig 2

Fig 1 Membrane potential changes in single frog muscle fiber (upper traces) in response to linearly rising currents of different gradients (lower traces) Five superimposed recordings fall of stimulus current and membrane repolarization after break of current not shown Current thresholds as indicated by gaps in recordings 0 24 0 22, 0 20 0 17 and 0 14 μA Time from onset of stimulation

Fig 2 To illustrate determination of the critical membrane potential at an arbitrarily chosen stimulus gradient (3 7 μA/sec) Stepwise increase in duration of linearly rising current Only final phases of the superimposed stimulus recordings and membrane potential changes are shown and time is given from onset of stimulation Current fall time 10 msec small downward artifact in potential recording at break of current Resting potential —86 mV Full description in text

values are reduced as the current gradients are lowered The results of a close analysis of firing level, stimulus threshold and spike configuration using stimulus gradients over a range from 0 4 to 400 μA/sec will be presented below

The rate of rise of the current was calculated from the slope of the current recordings It is however not easy to visualize the velocity of the activation processes on the bas...

sec

In view of the gradual change of the membrane potential into the rising phase of the spike during a slowly rising stimulus it is difficult exactly to determine the level at which the spike takes off, in the present study this has been done by the following method A current of a given gradient was stepwise increased in strength and the corresponding membrane potential changes recorded as shown in Fig 2 As appears from the superimposed recordings the weakest stimuli applied were below threshold for spike discharge but after a small further increase in current strength a spike was initiated, as also after the subsequent slightly suprathreshold stimuli By increasing the duration of the linearly rising stimulus by small steps the critical firing potential as well as the current threshold at the chosen gradient could be determined fairly accurately, the margin between a depolarization not inducing and one inducing, spike discharge could usually be limited to 1—2 mV In the experiment

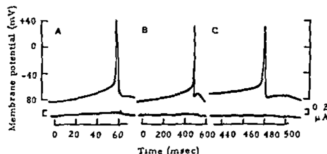


Fig 3 Comparison of responses obtained at two different stimulus gradients resulting in spike discharges at latencies of about 60 msec (*A*) and 480 msec (*B*) *C* same stimulus gradient as in *B* but recorded at fast sweep speed (same as in *A*) showing only final phase of membrane potential change Time from onset of stimulation

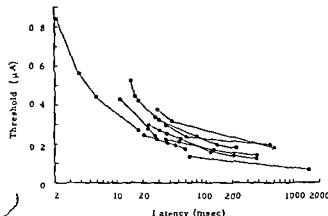


Fig 4 Stimulus thresholds at various linearly rising current gradients plotted against latency of spike response (log scale) Values from each of the nine different fibers tested connected by lines Resting potentials from —86 to —89 mV

Fig 2 the critical membrane potential, determined as the membrane potential value at the largest depolarization not resulting in spike discharge, was —50 mV, and determinations made at the same stimulus gradient on eight different fibers of the same muscle gave a mean value of —50 mV (range —48 to —52 mV) This agrees reasonably well with corresponding values previously obtained by square wave stimulation in experiments on frog sartorius muscle (Fatt and Katz 1951, Jenerick 1956) Since this method requires repeated stimulation for each determination of the firing level it was limited to studies of only two different gradients in each fiber and to gradients resulting in latencies less than 300 msec Experiments performed on twenty fibers showed that over this range of gradients the firing level in a particular fiber varied only within the errors of the method of determination

The firing level variation at current gradients over a wider range, corresponding to latencies from 10 msec up to about 1 sec, was estimated approximately by photographic projection of the potential curves obtained from single fibers at different stimulus gradients using the same sweep speed (*cf* Fig 3) Coincident curves at the foot of the spikes were taken as evidence against major changes in firing level The variations in firing level as estimated by this method should not exceed 5 mV within a range of gradients corresponding to spike latencies up to about 1 sec

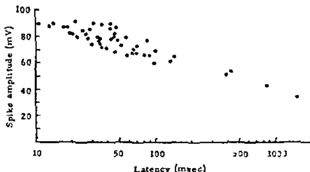


Fig 5 Spike amplitudes at different stimulus gradients plotted against spike latency (log scale) Amplitudes measured from critical firing level to peak of spike All determinations made in different fibers with resting potentials from —86 to —90 mV

The stimulus threshold was defined as the amplitude of the linearly rising stimulus current at the moment of spike discharge That a lowering of the stimulus threshold with decreasing gradient, as illustrated in Fig 1, occurs over a wide range of gradients is shown in Fig 4, in which the stimulus threshold has been plotted against spike latency on a logarithmic scale Since only a few stimuli were applied to each fiber, nine different fibers were used to cover the whole range of gradients As also appears from Fig 4, the absolute values of the stimulus threshold at one and the same latency vary in different fibers even though the variations in critical membrane potential are negligible (*cf* above), this is most likely due to variations in the effective resistance of the fibers tested (*cf* Fatt and Katz 1951)

In the experiment illustrated in Fig 1 the stimuli of the two steepest gradients give spikes of equal amplitude, as the gradients are lowered, moderate amplitude reductions occur which however need not be attributed to the difference in stimulus gradient but may also be due to fatigue since these stimuli were the last to be applied in the experiments (*cf* Jenerick 1959) A similar relation between stimulus gradient and spike amplitude was however invariably observed also when starting with the lowest gradient even though the difference in amplitude was usually more pronounced in experiments starting with the steepest gradient

In one series of experiments only one stimulus was applied to each fiber in order to eliminate fatigue phenomena The results obtained are shown in Fig 5, in which the spike amplitudes measured from critical membrane potential to peak of spike have been plotted against latencies Since the spike amplitude varies somewhat with the resting potential, which ranged from —86 to —90 mV in the fibers tested, comparisons of the spike amplitudes at different latencies have to be made with a certain caution It is evident, however, that a maximum amplitude of 90 mV could be attained as long as the latency was below 40—50 msec and that within this range the variations in spike height were moderate and could in many cases be referred to differences in the resting potentials At longer latencies on the other hand, the spike amplitude was invariably reduced The spike duration as measured at the foot of the spike remained unchanged over the range of latency variations tested

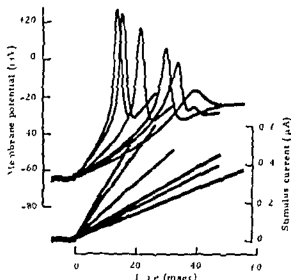


Fig. 6. Superimposed recordings of graded responses obtained at linearly rising currents of different gradients in a depolarized muscle fiber (resting potential −65 mV). Current fall and repolarization after current break not shown. Time from onset of stimulation.

Responses of depolarized fibers

Five per cent of the fibers studied had resting potentials ranging between −60 and −85 mV, i.e. lower than normal, and their responses to linearly rising currents differed from those of the normal fibers. A typical response pattern is shown in Fig. 6. In this fiber (resting potential −65 mV) the spike elicited by the steepest gradient closely resembled that evoked in a normal fiber but as the gradient was lowered there was a pronounced reduction in amplitude and increase in duration of the responses and at the lowest gradient only a "hump" resulted. At a still lower gradient (not shown in the figure) also this hump disappeared and the curve levelled off. To be able to generate a spike the rate of rise of the current must thus exceed a certain minimal gradient. These characteristic changes in the depolarized fiber occurred at gradients corresponding to spike latencies below 40 msec. In the normal fiber there was no measurable change in spike duration within this range and only a minor amplitude variation. Depolarization can thus be said to convert an all-or-none spike response into a graded response.

As appears from Fig. 6 the transition from slow subthreshold depolarization to spike potential is more gradual than in the normal fibers, and hence it was impossible to define a take-off level of the spike. It also proved to be difficult to determine a firing level by the method illustrated in Fig. 2 since the excitability of the depolarized fibers is so unstable that their responses to repeated stimulation tend to be continually varying. For similar reasons accurate determinations of the stimulus threshold were difficult to perform in depolarized fibers.

Comparisons of the response patterns of fibers at different degrees of depolarization suggested that the lower the resting potential the greater was the tendency to increased duration and reduced amplitude of the spike response. In order to study

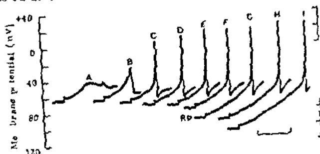


Fig 7 Muscle fiber responses to linearly rising current of constant gradient (2 8 μA sec) after pre setting the membrane potential at different levels below (A–F) and above (H–I) the resting potential (—89 mV marked RP) by polarizing current

the relationship between the response to a linearly rising current and the membrane potential level a series of experiments was performed in which the membrane potential of normal muscle fibers was pre set at various levels above and below the resting potential by application of constant current across the membrane A typical experiment is illustrated in Fig 7, which shows the responses to stimuli of an arbitrarily chosen gradient, in record G at the resting potential of the fiber (—89 mV marked RP), in H and I after hyperpolarization to two different levels and in A–F after varying degrees of depolarization The initial horizontal part of each recording represents the membrane potential level before onset of the linearly rising current The only change apparent in H and I is that a larger depolarization is necessary to attain the critical membrane potential the spike amplitudes being the same as in G In A–F on the other hand the response amplitude is reduced and more so the larger the preceding depolarization only a "hump" resulting from the current pulse in A

Subthreshold processes and spike activation

Studies of local subthreshold phenomena during square wave stimulation of frog muscle fibers have revealed the presence of two opposing processes Jenerick 1959 The depolarizing process which becomes apparent as a deviation in positive direction from the potential change due to passive discharging of the resting membrane is dependent on the presence of extracellular sodium ions and has been attributed to an increase in sodium conductance In the absence of external sodium there is during sufficiently strong stimulation a deviation from the passive polarization curve in the opposite direction and Jenerick a[illegible] this repolarizing process to an increase in potassium conductance [illegible] may however also be involved e g changes of the chloride [illegible] Boyle and Conway 1941 Hodgkin and Horowicz 1959

The time dependence of the [illegible] threshold processes was studied by Knutsson (1964 using linearly rising currents of different gradients The main results of his analysis are schematically summarized in Fig 8 The tracing refers to the actual membrane potential changes recorded during stimulation at three different gradients and the broken lines the potential changes to be expected from a passive membrane polarization The difference between the actual and the passive trem

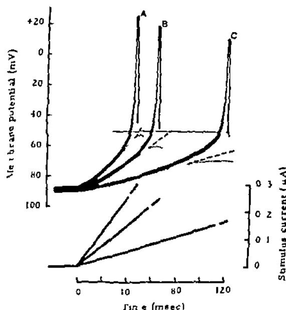


Fig 8 Membrane potential changes (upper traces A B and C) induced by three successively lower stimulus gradients (lower traces) Critical firing level indicated by thin horizontal line and current threshold by gaps in stimulus recordings Broken lines show the passive membrane potential changes to be expected from the current applied across the membrane The upward deviations of the actual membrane potential from these lines indicate a depolarizing activation which starts at different membrane potential levels in A B and C The dotted lines show the typical deviations of the membrane potential in negative direction occurring in fibers when the external solution is deprived of sodium and indicating a repolarizing activation

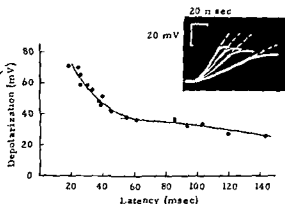


Fig 9 Muscle fiber in sodium free solution *Inset* Membrane potential changes obtained at four different gradients in a muscle fiber after replacing external Na by choline Broken lines represent expected passive membrane potential changes the points of departure of the actual potential curve from these lines show onset of the repolarizing activation process Resting potential 89 mV *Plotted curve* depolarization required to turn on the repolarizing activation process at different stimulus gradients plotted against latency of this activation Broken line indicates mean depolarization necessary to attain the critical firing level in fibers in normal Ringer's solution

brane potential changes is due to the depolarizing process and represents the active subthreshold potential which starts at a less depolarized potential level and attains a higher amplitude the lower the stimulus gradient After replacement of external Na by choline this activation process does not appear, instead the membrane potential deviates in the opposite direction, as schematically shown by the dotted lines in the figure

The time relations of this repolarizing process were studied more extensively in the present investigation and it was found that this process sets in at a longer latency at all gradients tested than does the depolarizing process a result consistent with Jenerick's (1959) observation that the repolarizing process has a larger time constant than has the depolarizing process It could also be established that the repolariz

ing process, like the active subthreshold potential, starts at a less depolarized membrane potential level the lower the stimulus gradient (cf Fig 8) Illustrative examples of actual curves obtained by stimulation of one and the same muscle fiber at four different gradients are given in the inset of Fig 9 The plotted curve in the figure is based on data from three experiments of the same type representing a wider range of gradients From this curve it becomes apparent that at the steepest gradients a depolarization of up to 70 mV is required to turn on the repolarizing process and that this process sets in at a lower depolarization the lower the stimulus gradient, a depolarization of 30 mV being sufficient to switch on the repolarizing process at the lowest gradient

The broken horizontal line in the curve indicates the average depolarization required for spike activation under normal ionic conditions In this connexion it is noteworthy that the resting potential of the fibers tested in sodium free milieu was about the same (—89 to —90 mV) as in normal ionic milieu If we presume that the repolarizing process develops in the same manner in fibers in normal and in sodium free milieu, then the curve demonstrates that at steep gradients the repolarizing process will not be switched on until the depolarization exceeds that required for spike initiation whereas at lower gradients, corresponding to spike latencies of more than 60 msec, the repolarizing process will start before the depolarization has reached threshold for spike discharge Reverting to Fig 8 we will find that in *A* the repolarizing process does not set in until in the course of the ascending phase of the spike, whereas in *B* and *C* it sets in during the subthreshold depolarization The fact that the spike amplitudes are reduced in *B* and *C* as compared to *A* and more so the more advanced the repolarizing process, favors the concept that the repolarizing process influences the size of the spike

This concept gains further support from the studies of the corresponding processes in depolarized fibers The fact that in muscle fibers with normal membrane potential a spike is elicited even at very low gradients, corresponding to latencies of about 1 sec, implies that the depolarizing process is predominant at the critical potential level under normal conditions In initially depolarized fibers on the other hand no response is obtained when the current rise is slower than a certain minimal gradient an obvious explanation would be that the repolarizing process then becomes powerful enough to counteract the depolarizing process A preliminary series of experiments indicates that this is in fact one mechanism behind the behavior of depolarized fibers Thus, in a series of experiments in which the membrane potential of fibers in sodium free milieu was pre set at different levels and the potential changes compared during stimulation at one and the same stimulus gradient it was found that the repolarizing process sets in at a higher membrane potential value the more depolarized the initial state of the membrane Such a change of the turning on of the repolarizing process in depolarized fibers should result in an augmentation of the counteraction on the spike depolarization A predominance of the repolarizing activity when the firing level is attained would explain the absence of spike responses at very low gradients

Discussion

The membrane responses to linearly rising currents during intracellular stimulation and recording have previously been studied only in motoneurons (Araki and Otani 1959, see also Sasaki and Otani 1961 Frank and Fuortes 1960 Bradley and Somjen 1961) In such experiments the level of displacement of the membrane potential at the moment of spike initiation could not be determined since the stimulus current through the recording electrode caused artifact distortions Such artifacts are negligible in studies of the muscle fiber which permits the insertion of separate electrodes for stimulation and recording, and isolated muscle is thus a suitable preparation for absolute measurements of the membrane potential changes during application of linearly rising currents

In muscle fibers with normal resting potential the critical firing level as determined by the exact method of stepwise increasing the stimulus strength did not show any significant variations at gradients corresponding to latencies up to 300 msec For the lowest gradients tested corresponding to latencies between 300 msec and about 1 sec this method of determination could not be applied (*cf* Results above) but comparisons of take off levels indicated that even at those gradients there were no conspicuous changes in the critical firing level Fatt and Katz (1951) using square wave stimuli of amplitudes just below or just above threshold also found that the level at which the spike originated was independent of the time taken to reach it their results refer however only to responses of latencies between 0 3 and 8 msec

The fact that less current strength is required for spike generation the lower the stimulus gradient although the critical membrane potential remains virtually constant may be explained in part by the time constant of the membrane and in part by changes in the subthreshold activation Owing to the comparatively large time constant of the muscle fiber membrane about 35 msec according to Fatt and Katz (1951) the voltage current ratio across the membrane becomes larger the slower the rise of the current when the gradient exceeds about 0 2 μA sec (Knutsson 1964) Since all gradients used in this investigation were steeper stimuli of a given amplitude were more effective the slower the current rise in other terms to attain the same passive membrane polarization less current strength is required the slower its rise As illustrated schematically in Fig 8 a lowering of the stimulus slope results in an increase of the active subthreshold depolarization implying that a larger proportion of the depolarization to the critical firing level is brought about by the activation processes and this would also result in a lowering of the stimulus threshold

A similar reduction of the stimulus threshold as the rate of current rise is lowered has been observed in spinal motoneurons at short latencies whereas at long latencies the stimulus threshold was found to rise as the rate of rise of the stimulus was lowered (Frank and Fuortes 1960 Bradley and Somjen 1961) In muscle fibers with normal resting potential no such threshold rise could be observed at the gradients used by us but might probably have occurred if still lower stimulus gradients had been applied

The most striking feature of the muscle membrane properties revealed in the present investigation is the characteristic change in response pattern resulting from even a small decrease of the resting potential While in normal fibers a lowering of the stimulus gradient has but a moderate effect on the spike height and none at all on its duration the effect on both these parameters is pronounced in the depolarized cell the spike being gradually reduced until only a hump like potential occurs just before a minimal gradient is reached at which no spike is generated

That the spike amplitude is reduced when muscle fibers are depolarized is known from experiments using instantaneously rising currents (Benoit and Coraboeuf 1955 from the present investigation it is evident that when the spikes are elicited by slowly rising currents this amplitude reduction becomes more pronounced and simultaneously the spike duration increases, resulting in a graded response Thus the time dependent activation processes in the membrane seem to be very susceptible to changes in the membrane polarization

Similar changes in spike height and duration with depolarization have been observed in spinal motoneurons (Kolmodin and Skoglund 1958 1959 and these neurons resemble muscle fibers also insofar as a minimal gradient could usually not be defined at normal resting potential but was observed in depolarized cells (Bradley and Somjen 1961) In this connexion it is noteworthy that a minimal gradient is usually not found in nerve fibers with intact circulation (Skoglund 1942 but can be demonstrated after interruption of the blood supply (Parrack 1940) as well as in isolated nerve fibers (Tasaki 1950 Diecke 1954) which are liable to depolarize

On account of the gradual transition from subthreshold potential to spike discharge in depolarized fibers it was not possible to judge whether the critical firing level and threshold in these fibers varied with the stimulus gradient A gradual rise in threshold is, however, likely to occur as the stimulus gradient approaches the minimal gradient at which excitation is completely abolished such a rise in stimulus threshold with lowering of the rate of rise of the current has in fact been observed in toad muscle fibers exhibiting a minimal gradient (Lucas 1907)

The role of the subthreshold repolarizing process as a factor determining the spike amplitude in normal and depolarized muscle fibers has been discussed above see Results) According to the ionic theory another process influencing the amplitude of the nerve action potential is the inactivation of the sodium-carrying system (Diecke 1954 Vallbo 1964) As far as muscle fibers are concerned no data are available about this process but it is noteworthy that as appears from Fig 8 the active subthreshold potential preceding the spike is of longer duration and reaches a higher amplitude the lower the stimulus gradient if this change in subthreshold potential is due to a more pronounced Na activation this may imply that also the Na inactivation becomes larger cf Hodgkin and Huxley 1952 which should result in a reduction of the spike amplitude Considering the complex ionic exchanges that apparently occur in muscle fiber cf Adrian and Freygang 1962 and the lack of data on the actual current flow during muscle excitation a correlation of the present results to ionic events can however only be tentative

Summary

1 The spike responses of frog muscle fiber to linearly rising current were studied using intracellular microelectrodes for stimulation and recording The rate of rise of the current was varied between 0 4 and 400 μA/sec, resulting in spike latencies ranging from 2 msec up to about 1 sec

2 In muscle fibers with resting potentials within the normal range (—86 to —90 mV) the critical firing potential was not significantly altered when the stimulus gradient was changed Direct measurements of the membrane potential at the largest depolarization not resulting in spike discharge gave a mean value of —50 mV (range —48 to —52 mV) and showed no significant variations when made in one and the same cell at various stimulus gradients resulting in spike latencies between 2 and 300 msec When comparing the take off levels at spike latencies up to about 1 sec the variation in critical firing level was estimated not to exceed 5 mV The stimulus threshold, *viz* the amplitude of the linearly rising current at spike discharge, decreased with lowering of the gradient

3 In muscle fibers with low resting potentials (—60 to —85 mV) as well as in normal fibers depolarized to the same values by application of constant current a graded type of spike response was obtained provided that the rate of the stimulus exceeded a certain minimal gradient These responses were characterized by a pronounced reduction in spike amplitude and increase in spike duration as the stimulus gradient was lowered

4 A schematic diagram of the subthreshold processes occurring in muscle during stimulation with slowly rising currents, based in part on earlier data is presented and an attempt made to correlate the spike responses in normal and depolarized fibers to these subthreshold processes

Support for this investigation has been provided by the foundation Gustaf och Fyra Svenssons Minne

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Acta physiol. scand. 1966, 68, 206—214

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Uptake, Storage and Release of Histamine by Rat Peritoneal Mast Cells in vitro

By

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Received 22 March 1966

Abstract

CABUT, M and Ö HAEGERMARK *Uptake, storage and release of histamine by rat peritoneal mast cells in vitro* Acta physiol scand 1966 *68* 206—214

Rat peritoneal mast cells when incubated in histamine H³ containing medium were shown to take up and concentrate h[illegible] cellular histamine concen[illegible] The exogenous histamine [illegible] ne was taken up [illegible] 5 HT inhibited

Schayer (1956 showed that rat peritoneal mast cells are able to decarboxylate histidine into histamine, which is then stored in the cells An important question is whether such cells also are able to take up and store preformed histamine It has been stated that *in vitro* neoplastic mouse mast cells not only synthesize and store histamine but take up exogenous histamine (Day and Green 1962, Day and Stockbridge 1964 While the present investigation was in progress, Furano and Green (1964) reported that normal rat mast cells also were able to take up extracellular histamine

In this paper the uptake binding and release of exogenous histamine by isolated peritoneal mast cells have been studied *in vitro* The experiments were designed to determine whether histamine is actively or passively taken up by the cells, whether it is then bound to the mast cell granules, and whether it can be released by the histamine liberator compound 48 80

Methods and Materials

Preparation of cells

Rat peritoneal mast cells were isolated by density gradient centrifugation in Ficoll® as described by Uvnäs and Thon 1959 Male rats of the Sprague Dawley strain weighing 200—400 g were used A buffered salt solution containing NaCl 154 mM KCl 2 7 mM CaCl₂ 0 9 mM Na₂HPO₄

* Visiting scientist Nov 196[illegible] Dec 19[illegible]

40 mM KH_2PO_4 2 7 mM, glucose, 1 mg/ml, human serum albumin 1 mg ml (pH 7 0 was used throughout the experiment i e for perfusion of the peritoneal cavity washing of the cell and as incubation medium For perfusion the solution also contained heparin 10 IU ml After isolation and washing procedures the cells were pooled and counted in a Burker chamber

Incubation technique

The pooled cells were divided into equal aliquots in centrifuge tubes centrifuged at 350 t 3 min and the supernatants carefully decanted The cells were then resuspended and incubated at 37° C in 0 5 ml of buffered salt solution to which H^3 labelled histamine had been added Th number of cells in each tube varied in different experiments between 0 3 10^6 and 0 8 10^6

significantly exceed the background

Histamine was extracted from the cells by adding 1 5 ml of 0 1 N HCl and heating n a boiling water bath for 3 min Aliquots of the extracts were taken for radioactive measurement histamine assay and in some experiments for chromatography

Analysis

Radioactivity was measured in a Tri Carb® liquid scintillation spectrometer Model 314 EX 2 (Packard Instrument

almost identical results when run parallel All histamine values are expressed as free base

u and acetyl histamine

Liquid scintillation technique was applied also when measuring the radioactivity of the chromatograms One centimeter strips of the dried chromatograms were put into the counting vials containing 15 ml of the scintillation fluid Radioactivity was counted directly without any extraction procedure (Wang and Jones 1959)

Materials

Histamine H^3 d hydrochloride, 1 0 Ci/mmole (Schwarz Bio Research Inc Orangeburg N Y U S A)

Ficoll®

Results

Table I shows the data obtained from two experimental series where mast cells were incubated with histamine-H^3 1 1 μg ml and 1 4 μg ml for 60 and 160 min respectively Histamine uptake amounted to 2 9 and 6 8 μg 10 ^{3} 10^6 cells which corresponds respectively to 0 018 and 0 045 per cent of the total histamine content in the cells after incubation The ratios of intracellular extracellular histamine H^3 concentrations were 2 8 and 4 7

TABLE II Effect of enzyme inhibitors on histamine uptake during 60 min Cells were preincubated with inhibitor for 15 min at 37° C and then histamine H^3 was added Values given as means and standard errors Figures in parentheses represent number of experiments

Inhibitor	Uptake in presence of inhibitor, as a percentage of uptake in controls
Potassium cyanide, 10^{-3}M	89 ± 9 (5)
2,4-dinitrophenol, 10^{-3}M	85 ± 34 (3)
Iodoacetic acid 10^{-3}M	70 ± 9 (4)
Ouabain, 10^{-3}M	90 ± 18 (3)

It is evident that the amounts released, 0 2—1 5 μg/10^6 cells, greatly exceeded the uptake under the corresponding conditions (Fig 1)

Effect of extracellular histamine concentration on uptake

The histamine uptake was measured after incubation of the cells for 60 min in different concentrations of histamine H^3, varying between 4 3 × 10^{-3} and 1 1 μg/ml (Fig 3) The uptake was directly proportional to the extracellular histamine concentration

To ascertain whether the cells could be saturated with histamine they were incubated for 160 min with very high concentrations of histamine To a constant concentration of histamine H^3 (12 5 μCi/ml = 1 4 μg/ml) different amounts of unlabelled histamine were added, the final histamine concentrations varied between 1 4 μg/ml and 602 μg ml The results, corrected for isotope dilution, are shown in Fig 4 The amounts taken up were directly proportional to the histamine concentrations in the medium except for the highest concentration, where the curve showed a tendency to level off

Temparature coefficient

Histamine uptake at different extracellular concentrations of histamine H^3 was measured at 27 C and 37 C after incubation for 60 min (Fig 3) Uptake was only slightly decreased when lowering the temperature by 10° C The temperature coefficients (Q_{10}) for the different histamine concentrations (except for the lowest concentrations) were 1 1 1 4 1 4 and 1 1 At the lowest concentration uptake was higher at 27° C than at 37 C This was probably due to the difficulties in measuring accurately the very small amounts taken up by the cells the counting rate only slightly exceeded the background

Effect of enzyme inhibitors on histamine uptake

Table II shows the effect of some enzyme inhibitors on histamine uptake during 60 min The cells were preincubated with the inhibitor for 15 min and then radioactive histamine, 1 1 μg/ml, was added In spite of the high inhibitor concentrations used there was only a slight decrease in uptake

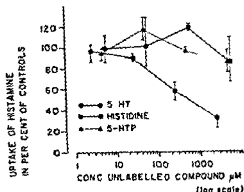


Fig 5 Effect of 5 HT, histidine and 5 HTP on uptake of histamine H³ 12 5 µM (1 4 µg ml) The cells were incubated at 37° C for 160 min 5-HT (3 expts.) Histidine (3 expts) 5-HTP (2 expts) Each point represents the mean uptake as a percentage of uptake in controls Vertical bars represent range

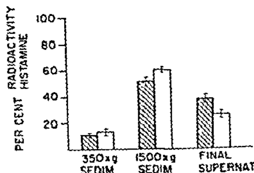


Fig 6 Subcellular distribution of radioactivity ▨ and of histamine □ in mast cells after incubation with histamine H³ The columns represent the means of 7 expts expressed as the percentage of total radioactivity and total histamine present in the cells Vertical bars represent standard errors

Influence of 5 HT, histidine, and 5 HTP on the uptake of labelled histamine

Rat mast cells contain also 5 hydroxytryptamine (5-HT) (Benditt *et al* 1955 which can be synthesized in the mast cell by decarboxylation of 5-hydroxytryptophan (5-HTP) (Hagen and Lee 1958, Lagunoff and Benditt 1959) Moreover, extracellular 5-HT has been shown to enter rat mast cells *in vitro* (Furano and Green 1964) To determine whether the presence of either these compunds or of histidine would influence the uptake of histamine, mast cells were incubated for 160 min with different amounts of unlabelled 5 HT, L 5 HTP and L-histidine in addition to the histamine H³ (12 5 µM = 1 4 µg/ml) When unlabelled 5 HT, in a final concentration of 250 or 2,500 µM, was added to the incubation medium the uptake of histamine H³ decreased (Fig 5) whereas the addition of the amino acids histidine or 5-HTP did not affect histamine uptake

Intracellular localization

After incubation in radioactive histamine the mast cells were washed 3 times, resuspended in 0 34 M sucrose and disintegrated by sonic oscillation at 1 0 × 10⁴ c s for 15—30 sec (Lagunoff and Benditt 1963) By differential centrifugation at 350 × g for 3 min and 1,500 × g for 45 min three fractions were obtained one con-

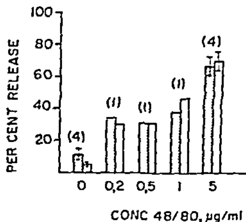


Fig 7 Release of radioactivity and histamine from mast cells (preincubated in histamine H³) induced by compound 48/80 Each column represents the mean release of radioactivity ▨ and histamine □ expressed as the percentage of total radioactivity and total histamine present in the cells The figures in parentheses denote the number of experiments Vertical bars represent standard errors

cell debris, coarse material and the largest granules (350 g sediment), another containing primarily mast cell granules (1,500 g sediment) and a third fraction (final supernatant) containing soluble material and the remaining subcellular components The distribution of radioactive histamine and of total histamine (endogenous + exogenous) was determined for the different fractions (Fig 6) Since the amount of exogenous histamine was very small in comparison with the endogenous the amount of total histamine can be considered approximately equal to that of endogenous histamine Thus, the distribution of exogenous and endogenous histamine in the cellular fractions was about the same

Histamine release by compound 48/80

Mast cells were incubated for 60 min at 37 C using 1 1 µg histamine H³ per ml After washing 3 times the cells were reincubated with the histamine liberator compound 48/80 5 µg/ml In one experiment different concentrations of 48/80 were used (0 2, 0 5, 1 0 and 5 0 µg/ml) After 10 min the incubation was stopped by centrifugation for 3 min at 350 × g Histamine released by 48/80 was recovered in the supernatant whereas the histamine remaining in the cell residue was extracted from the sediment by heating in a boiling water bath for 3 min in 0 1 N HCl After neutralizing with NaOH, total histamine and radioactive histamine were determined in the supernatant and the sediment. Radioactive histamine was released in the same relative amounts as the total histamine of the cells Fig 7

Discussion

It has been shown in the present investigation that isolated rat peritoneal mast cells *in vitro* are able to take up small amounts of histamine from the surrounding medium

The histamine uptake is apparently a passive process This conclusion is based on the following facts The histamine uptake was small and directly proportional to the extracellular histamine concentration Saturation was not obtained even

for very high extracellular histamine concentrations The temperature coefficients were low and metabolic inhibitors did not seem to influence the histamine uptake This is in agreement with the observations made on neoplastic mouse mast cells, where the histamine uptake was regarded as being passive (Day and Stockbridge 1964

In our studies with sonically disintegrated mast cells, 55—60 per cent of the endogenous and the exogenous histamine were recovered in the granular fraction Lagunoff *et al* (1964) reported a similar distribution using the same technique However, in intact mast cells all the endogenous histamine is probably stored in the granules Thus the extragranular histamine observed in the present experiments may be released during the cell disintegration and fractionation procedures Since the exogenous histamine taken up and the endogenous were bound to the granules in about the same proportion, it seems reasonable to conclude that all the exogenous histamine taken up was also stored in the granules of the intact cells This conclusion may be supported by the finding that the histamine liberator compound 48 80 which is known to cause extrusion of mast cell granules (Paton 1957) released both exogenous and endogenous histamine in the same relative amounts

In the present experiments the intracellular/extracellular histamine H^3 concentration ratio was higher than 1, indicating that the concentration of radioactive histamine was higher inside the cell than outside Furano and Green (1964) observed a concentration ratio of 9 2 after incubation of rat mast cells for 60 min in histamine C^{14}, 0 74 $\mu g/ml$ In the present study the ratio was 2 8 after incubation for 60 min in histamine H^3, 1 1 $\mu g/ml$ In the previous section it was concluded that the exogenous histamine taken up was mainly bound to the granules This implies that the concentration of free histamine in the cytoplasm was low Thus histamine need not necessarily be transported into the cell against a concentration gradient although the concentration ratio is > 1 but a passive downhill diffusion of histamine through the cell membrane into the cytoplasm is quite possible As soon as the histamine has entered the cell it may be rapidly bound to the granules

Like histamine, 5 HT is bound to the granular fraction of rat peritoneal mast cells (Haegermark unpublished) and in neoplastic mouse mast cells Hagen Barrnett and Lee 1959) The two cell types are capable of taking up 5 HT *in vitro* Furano and Green 1964 Day and Green 1962) It is not known if 5 HT is bound in the same way as histamine Day and Stockbridge 1964 found that equimolar concentrations of 5 HT inhibited the uptake of histamine by neoplastic mouse mast cells In the present investigation no inhibition was observed when the molar ratio between histamine and 5 HT was 1 2 whereas the histamine uptake was decreased to about 60 per cent when the molar ratio was 1 20 and to 30 per cent at the ratio 1 200 It is not possible to make a final conclusion as to the nature of the inhibition It might be due to cellular injury induced by the high concentrations of 5 HT Contrary to this however are the findings that the cells are still capable of taking up histidine and decarboxylating it into histamine in the presence of these high doses of 5 HT (Cabut and Haegermark to be published) Another possibility may be a competitive inhibition between histamine and 5 HT Since the present data indicate that hista

mine passively diffused into the cell, 5 HT should not competitively inhibit the passage of histamine through the cell membrane On the other hand, if exogenous histamine and 5 HT compete for the same binding sites in the cells, the histamine uptake would be inhibited by an uptake of 5 HT

In these studies, the presence of extracellular histidine did not influence histamine uptake When studying the uptake of the amino acid histidine H^3 by mast cells (Cabut and Haegermark, to be published) it was observed that histidine uptake was not inhibited by the presence of histamine in the medium These findings are consistent with the idea that there are different uptake mechanisms for histamine and histidine and/or different storage sites for exogenous and endogenous hista mine

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Acta physiol. scand. 1966. 68. 215—227

From the Laboratory for the Theory of Gymnastics University of Copenhagen Denmark

Regulation of Body Temperature and Heat Dissipation at Different Levels of Energy- and Heat Production in Man

By

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Received 22 March 1966

Abstract

NIELSEN, B *Regulation of body temperature and heat dissipation at different levels of energy- and heat production in man* Acta physiol scand 1966 *68* 215—227

In treadmill experiments the ratio between total heat production and metabolic energy production was varied by having a subject walking or running uphill horizontally or downhill at different speeds between 2 and 14 km/hr In the steady state of work of different intensities the body temperature (esophageal or rectal) was found to increase to levels dependent on the energy production but independent of the total heat production At constant environmental temperature the sweat rate and the skin circulation expressed as conductance of the peripheral tissues varied in proportion to the total heat production and seemed to be independent of the internal temperature It is concluded that the thermoregulation during work serves two purposes 1) the setting of the internal temperature at higher levels proportional to the oxygen uptake and 2) the regulation of the heat dissipation in such way that it equals the total heat production independent of the internal temperature level

In recent experiments (B Nielsen and M Nielsen 1965 b) it was found that the thermoregulatory responses produced by active heating, *i e* exercise on a Krogh bicycle ergometer, and by passive heating, through diathermia, were remarkably similar At the same rate of heat production (or of heat induction), and the same skin and rectal temperatures, the skin blood flow increased to the same level and the increase in sweat rates were not much different The rectal temperature increased to about the same value and was in both conditions nearly independent of environmental temperature These experiments therefore seemed to show that the main stimulus for the increase in heat dissipation during the steady state of work is the increased internal temperature caused by the increased heat production and that special "work factors" only to a minor extent influence sweat rate and skin blood flow

Unfortunately thermoelectric measurements of temperatures were not possible during diathermia The rectal temperature was determined by a mercury thermometer and was, therefore, possibly not as reliable as the thermoelectric measurements

in the work experiments The experiments further suffered in that the measurements of temperature could only be performed during small interruptions in the diathermia

The aim of the present study is to examine more closely the effect of a passive heating at higher rates of heat production and heat induction than could be obtained in the diathermia experiments The thermoregulatory responses were studied in conditions where the metabolic energy production and sum of the heat production and heat induction could be varied independently, i e during grade walking on a treadmill

Methods

The rectal temperature and the esophageal temperature were measured thermoelectrically the rectal temperature in four depths and the esophageal temperature just above the diaphragm as described earlier (B Nielsen and M Nielsen 1962) The measurements are considered to be accurate to about $\pm$ 0 025° C The steady state" values in the graphs are the average of the three measurements in the last 10 min of the experimental period

1937)

The respiratory gas exchange was measured by the Douglas bag method and samples of the gas were analysed in duplicate on the Scholander apparatus (Scholander 1947) The average of two determinations of gas exchange was used for calculating the energy production in each experiment

The heat production during the experiments was calculated according to the formula

$H = M \pm W$

where *H* is the *rate of total heat production in kcal/hr* *M* is the rate of energy production calculated from O_2 uptake (4 9 kcal per l oxygen) and W is the external work converted to heat in kcal/hr (Snellen 1960)

W was calculated as G v sin α $\frac{1}{427}$ kcal/hr where

G = body weight in kg
v = speed of walking (running) m/hr
α = inclination of the treadmill in geometrical degrees.

Procedure

the placing of the thermocouples
or running at the fixed speed and
s were made at intervals of about

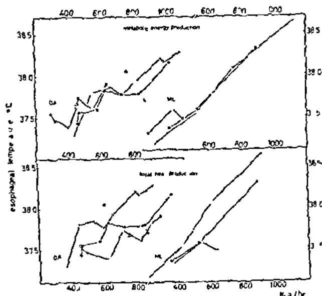


Fig 1 Esophageal temperature in the steady state of work in relation to the rate of metabolic energy production (above) and in relation to the rate of total heat production (below) Environmental temperature 21½° Two subjects OA and ML

Inclination of the treadmill { 0° (horizontal) ●——●; — 10° (downhill) ○——○; + 10° (uphill) +——+ }
In two experiments the inclination was + 15° ▲ and — 15° △

5 min After a preliminary period of 20 min of work he stepped off the treadmill and sat down on the seat of the Krogh balance for the first weighing This pause lasted 1½—2 min The subject returned to the treadmill and continued the work in the 45 min experimental period During the experimental period readings of rectal and esophageal temperatures were taken at 5 min intervals, and skin temperatures and room temperatures were measured 4 times Expired air was collected in Douglas bags after 25 min and 35 min work in the experimental period The pulse rate was counted at regular intervals

After 45 min the work was stopped and the subject was weighed again as quickly as possible that is less than 1 min after stopping

Thanks to the preliminary "warming up period heart rate skin blood flow ventilation O_2 uptake sweat secretion and esophageal temperature reached a steady state and skin temperature and rectal temperature came very close to a steady state level before the actual experimental period started The pause necessary for the weight loss determination, had only a negligible effect on the internal temperature but since the sweat secretion diminished so rapidly after cessation of work the weight loss would be slightly underestimated However the shortness of the pause as compared to the experimental period made this error minimal and nearly equal in all the experiments

Results

Fig 1 above shows the relationship between the esophageal temperature in the steady state of work and the rate of metabolic energy production in two subjects OA and ML. Below the esophageal temperature is plotted in relation to the total heat production (metabolic ± work)

The correlation between the esophageal temperature and the energy metabolism seemed to be the same for uphill, horizontal and downhill walking while the correla-

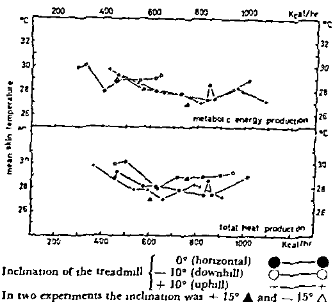


Fig 2 Mean skin temperature in the steady state of work versus metabolic energy production and versus total total heat production Environmental temperature 21 ½° Subject OA

Inclination of the treadmill { 0° (horizontal) ●——● ; — 10° (downhill) ○——○ ; + 10° (uphill) +——+ }
In two experiments the inclination was + 15° ▲ and — 15° △

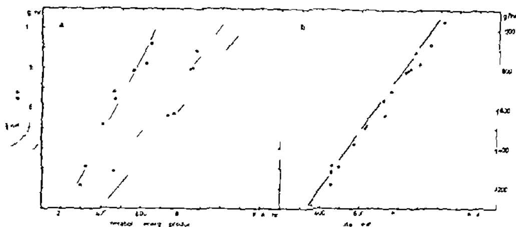


Fig 3 Sweat rate in relation to the rate of metabolic energy production (a) and sweat rate in relation to the rate of total heat production (b) Environmental temperature 21 ½ Subject OA

Inclination of the treadmill { 0° (horizontal) ●——● ; — 10° (downhill) ○——○ ; + 10° (uphill) +——+ }
In two experiments the inclination was + 15° ▲ and— 15° △

tion between the esophageal temperature and the total heat procuction was different for the three conditions

All the experiments were performed at an average ambient temperature of 21 ½° C (average of walls and air) The skin temperature was decreasing only about 1—2° C with increasing energy metabolism (Fig 2)

As shown in Fig 3 b and 4 b the sweat rate increased linearly with increase in *total heat* production The relationship between sweat rate and metabolic energy production, Fig 3 a and 4 a, however was different for negative work (downhill

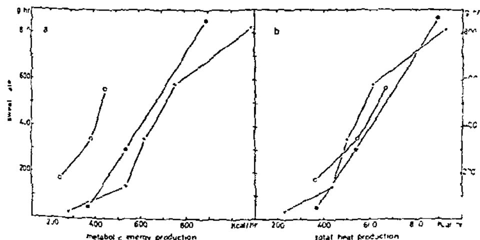


Fig 4 Sweat rate ... d sweat rate in relation to ... Subject ML.

Inclina ...

(+ 10° (uphill)

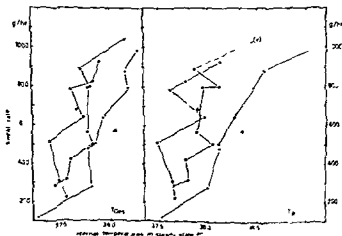


Fig 5 Sweat rate versus esophageal temperature (T_{Oes}) and rectal temperature (T_R) Environmental temperature 21 , Subject OA

Inclination of the treadmill { 0° (horizontal) 10° (downhill) + 10° (uphill)

In two experiments the inclination was + 15° ▲ and — 1

walking , walking on the level, and positive work uphill walking) At the ... gen uptake metabolic energy production) the rate of sweating ... walking downhill, and lower walking uphill, than when walking ...

Fig 5 and 6 shows the relationship between the sweat rate ... temperature At the same internal temperature (esophageal or ...

TABLE I The mean of the steady state values of internal temperatures (T_R and T_{Oes}) and the mean rates of energy production and heat production in three conditions 1) uphill work 2) horizontal work at an energy production equal to the uphill work 3) horizontal work at a total heat production equal to that obtained during uphill work 15 expts Subject ML

Incl of treadmill	Speed km/hr	O_2 uptake l/min STPD	Energy production kcal/hr	Total heat production kcal/hr	Mean T_{Oes}	Mean T_R
+10	6	2 80	823	675	38 10 SD 0 13 n=6	38 60 SD 0 17 n [illegible]
0°	13	2 82	829	829	37 91 SD 0 08 n 5	38 32 SD 0 11 n 5
0°	11	2 31	680	680	37 85 SD 0 07 n 4	38 28 SD 0 10 n=4

TABLE II The steady state values of esophageal temperature read on graphs like Fig 1 in three subjects at a rate of energy production of 600 kcal/hr and at a rate of total heat production of 600 kcal/hr The numbers in parentheses in the table gives the corresponding values of total heat production or energy production respectively

	Incl of tread mill	Esophageal temperature C		
		Subject OA	Subject OPK	Subject ML
Energy	10	37 75 (470)	37 75 (500)	37 70 (480)
Production	0	37 65 (600)		37 [illegible] (600)
600 kcal/hr	10	37 80 (900)	37 90 (850)	
Total heat	+10	37 85 (775)	38 05 (770)	38 05 (750)
Production	0	37 65 (600)		37 70 (600)
600 kcal/hr	10	37 55 (400)	37 60 (400)	37 55 (420
Number of expts in graph		24	8	12

levels obtained during horizontal running 1) at the same metabolic energy production (823 kcal/hr) and 2) at the same total heat production (M — external work) (675 kcal/hr) Table I shows that although the rate of total heat production was less during uphill walking the internal temperature was not lower in this condition The internal temperatures during uphill walking was significantly higher than the values obtained at the same total heat production during horizontal walking (running)

In Table II the esophageal temperatures obtained in steady state of uphill, horizontal and downhill walking are compared at a rate of energy production of

600 kcal/hr and at a rate of total heat production of 600 kcal/hr in three subjects The values of esophageal temperature were read on graphs relating esophageal temperature and respectively energy production and total heat production, *cf* Fig 1 In all three subjects the differences between the temperatures reached in the three conditions were largest compared at the same rate of total heat production The highest values were obtained when the energy production was the highest *i e* during uphill walking At the same rate of energy production the esophageal temperatures were nearly the same in the three conditions with unsystematic differences

Discussion

The experiments seem to show that the thermoregulation during work is operating for two purposes The setting of the internal temperatures at a higher level, and the establishment of an equilibrium between heat production and heat dissipation

The setting of the internal temperature

The present finding that during work the internal temperatures (T_R and T_{Oes}) increase to new levels, the increases being proportional to the metabolic rates, is in agreement with earlier findings of M Nielsen (1938), Robinson (1949) B Nielsen and M Nielsen (1962) and many others

In these earlier studies it was established that the level of internal temperature is independent of the ambient temperature within wide limits, but depends on the work intensity

Normally work intensity, metabolic rate, and heat production vary in proportion to one another and to the changes in internal temperature However, in our experiments, where the metabolic rate and the total heat production change out of step, it was demonstrated that the work temperature level at a given metabolic rate is independent of the heat production too (Table I and II and Fig 1) This is a direct confirmation of the theory of Nielsen (1938) that the increased temperature during work is not a passive rise, caused by the increased heat production, but is due to a "setting of the body thermostat" at a higher level The temperature increase would benefit the enzymatic reactions in the working muscles and increase the efficiency of the work (Asmussen and Boje 1945)

This setting of the higher work temperature level in proportion to the energy production must be induced by some factor related to the working condition and, according to our experiments, independent of the heat production Such work factors ' have first been considered for the explanation of the regulation of respiration during work Krogh and Lindhard, (1913) proposed the theory that the work factor" was an 'irradiation" of impulses from the motor cortex to the respiratory centre Asmussen, Nielsen and co-workers (1943) found it more likely that afferent impulses from the working muscles were responsible for the increase in ventilation In a recent work (Asmussen *et al* 1965), with partial nervous block by curare, they suggested that the mechanism for the increased ventilation during work could be the feedback to the reticular formation of *afferent* impulses from the muscle spindl

Similar mechanisms have been considered for the regulation of temperature and sweating For instance Kitzing and Bleichert (1965) also used partial neuromuscular blocking with curare in a study of thermoregulation during exercise However, the increase in motor nerve activity, which presumably must occur during continued work when a certain part of the muscle fibers are blocked by curare, caused no deviation in deep esophageal temperature from pre infusion values

In the present treadmill experiments the summated mechanoreceptor output must be much greater during negative work than during positive work at the same energy production because the speed of the movements is about 3—4 times greater during downhill walking (Fig 8) and the muscle tensions must have been the same In this case it is unlikely that the thermoregulatory centre obtains information for the temperature setting through neuromuscular reflexes elicited from mechanoreceptors or by irradiation of cortical impulses However the adjustment of the temperature level could perhaps be obtained by means of central or peripheral receptors for metabolites occurring in proportion to the metabolic rate

Asmussen and Nielsen (1947) observed that the rise in rectal temperature during bicycling at a rate of 1100 kpm/min was the same and the O_2 uptake equal at sea level and at a lowered barometric pressure corresponding to an altitude of 4000 m although the pulse rate ventilation and degree of anaerobiosis was much greater at low pressure Further the rise in esophageal temperature during arm work and work with the legs must have been approximately the same at the same O_2 uptake estimated from results from Asmussen and Nielsen (1947) and B Nielsen and M Nielsen (1962) The degree of anaerobiosis was much higher during arm work which together with the low pressure experiments excludes lactic acid and local H from being the work factor in thermoregulation

Summing up it seems that the increase of the internal temperatures during work is brought about by some work factor possibly of chemical nature liberated by or in proportion to the extent of the oxydative processes This induces a change in the setting of the human thermostat perhaps in a manner similar to the action of a pyrogen in fever (Minard and Copman 1963 Cooper *et al* 1964) although it is not the same substances as is demonstrated by the ineffectiveness of salicylates on the work fever (Dawney and Darling 1962)

The regulation of heat dissipation

The maintenance of the body temperature at any level is possible when equilibrium between heat production and heat dissipation can be established Therefore when at a certain metabolic rate the rate of heat production is changed by adding or subtracting external energy (walking downhill or uphill) the heat surplus or deficit must be compensated by an equal change in the rate of heat dissipation Otherwise a temperature equilibrium is not possible

In this study the environmental temperature was the same in all experiments The mean skin temperature was almost the same too decreasing 1—2 at increasing energy production (Fig 2) The conditions for non evaporative heat loss therefore

are almost the same (Fig 8) Consequently, when a certain am int of energy given off to the treadmill (in uphill walking), or induced into the body during d hill walking), the heat loss through sweating must vary in proportion to t amount of heat produced in the body

That this actually happens can be seen in Fig 3a, 4a and 7 below At th energy metabolism (and internal temperature, Fig 1) the sweat rat and culation are much higher during negative work where extra heat is d lower during positive work, where heat is lost as external work On th the sweat rates and skin circulations are equal when compared at the rate total heat production (Fig 3b, 4b and 7 above), in spite of differer metabolism and internal temperature (Fig 5 and 6) This very nice the finding that passive heating, through diathermia at the same rate of tion or induction, produced the same sweat rate and skin circulation a

Recently Benzinger (1959) advanced a theory according to which the in temperature from a fixed "set point" of the thermosensitive areas in thalamus alone determines the rate of sweating Hammel *et al* (1963 pro an alternative to Benzinger's theory, that the "set point" of the temperatu e ing mechanism could change, because they found that the hypothalamic temperat of a dog shivering in the cold could be higher than when it was panting in h environments The change in "set point", they suggest, could be produced by im pulses from thermoreceptors in the skin, in the core of the body and from other factors, such as state of wakefulness and perhaps also muscular work Jackson and Hammel (1963) report that the "set" temperature seems to decrease during exercise in the dog and that perhaps also the sensitivity to temperature changes of the thermoregulatory system increases

In the treadmill experiments the esophageal temperature is the same at the same energy production in the steady state of uphill, horizontal and downhill work Fig 1) Also the average skin temperature was nearly equal in all the experiments Fig 2, so that the nervous input from the skin receptors must have been almost the same in the 3 conditions The esophageal temperature is considered a good index of the aortic blood temperature (Cooper and Kenyon 1957) It is thus hard to believe that the hypothalamic temperature should differ under these circumstances, and that the hypothalamic or skin temperatures should be responsible for the differences in sweat rate and skin circulation found when the rate of heat productions were different

Robinson (1962, 1963) and Robinson and co-workers (1965) suggested that neuromuscular reflexes take part in the regulation of sweating and skin circulation during work, but they found it less likely that these reflexes were elicited from mechanareceptors, and assumed that thermoreceptors, in the muscles were a more likely source

It would be interesting to measure muscle temperature during positive and negative work to see if the local temperature were higher during negative work even when the rectal temperatures were equal (Fig 5 and 6), thus providing the extra stimulus for the sweating mechanism and the skin circulation

Similar mechanisms have been considered for the regulation of temperature and sweating. For instance, Kitzing and Bleichert (1965) also used partial neuromuscular blocking with curare in a study of thermoregulation during exercise. However, the increase in motor nerve activity, which presumably must occur during continued work when a certain part of the muscle fibers are blocked by curare, caused no deviation in deep esophageal temperature from pre-infusion values.

In the present treadmill experiments the summated mechanoreceptor output must be much greater during negative work than during positive work at the same energy production because the speed of the movements is about 3—4 times greater during downhill walking (Fig. 8) and the muscle tensions must have been the same. In this case it is unlikely that the thermoregulatory centre obtains information for the temperature setting through neuromuscular reflexes elicited from mechanoreceptors or by "irradiation" of cortical impulses. However, the adjustment of the temperature level could perhaps be obtained by means of central or peripheral receptors for metabolites occurring in proportion to the metabolic rate.

Asmussen and Nielsen (1947) observed that the rise in rectal temperature during bicycling at a rate of 1100 kpm/min was the same and the O_2 uptake equal at sea level and at a lowered barometric pressure corresponding to an altitude of 4000 m, although the pulse rate, ventilation and degree of anaerobiosis was much greater at low pressure. Further, the rise in esophageal temperature during arm work and work with the legs must have been approximately the same at the same O_2 uptake, estimated from results from Asmussen and Nielsen (1947) and B. Nielsen and M. Nielsen (1962). The degree of anaerobiosis was much higher during arm work, which together with the low pressure experiments excludes lactic acid and local H⁺ from being the "work factor" in thermoregulation.

Summing up, it seems that the increase of the internal temperatures during work is brought about by some work factor, possibly of chemical nature, liberated by or in proportion to the extent of the oxidative processes. This induces a change in the setting of "the human thermostat", perhaps in a manner similar to the action of a pyrogen in fever (Minard and Copman 1963, Cooper *et al.* 1964), although it is not the same substance, as is demonstrated by the ineffectiveness of salicylates on the "work-fever" (Downey and Darling 1962).

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The maintenance of the body temperature at any level is possible when equilibrium between heat production and heat dissipation can be established. Therefore, when at a certain metabolic rate the rate of heat production is changed by adding or subtracting external energy (walking downhill or uphill), the heat surplus or deficit must be compensated by an equal change in the rate of heat dissipation. Otherwise a temperature equilibrium is not possible.

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Acta physiol scand 1966, 68 228—230

From the Institute of Occupational Health Tyoterveyslaitos Helsinki Finland

Effect of Ethionine on the Absorption of Palmitic Acid-1-C^{14} in the Rat

By

Esko Karvinen and Matti Miettinen

Received 30 March 1966

Abstract

Karvinen, E and M Miettinen *Effect of ethionine on the absorption of palmitic acid 1 C^{14} in the rat* Acta physiol scand 1966 *68* 228—230

Ethionine administration to female rats resulted in significantly lower rate of absorption of ingested palmitic acid 1 C^{14} as compared with that of saline treated controls

It has been implied that ethionine may facilitate fat absorption since increased chylomicron counts were noted in rats with ethionine induced fatty livers (Tidwell 1956) In earlier studies from this laboratory, it has been found that ethionine administration to female rats resulted in an increase in the radioactivity of the chylomicron fraction after ingestion of cholesterol 4 C^{14} (Karvinen and Miettinen 1962) but no such increase was noted after ingestion of palmitic acid 1 C^{14} (Karvinen *et al* 1964)

In the present paper, studies on the absorption of palmitic acid 1 C^{14} in the female rat are reported

Material and Methods

Female rats of the Wistar strain weighing 200 to 250 g were caged individually and given a [illegible] diet for 3 weeks *ad libitum* During the experiment the rats were fed with graham flour and water [illegible] were given *daily 3 intraperit* [illegible] [illegible] at 9 a m 3 p m and 10 p m On [illegible] ine were given daily at 9 and 3 p m

TABLE I Radioactivity of the feces and intestine serum lipid and liver fat of th ethionine and sal rats 28 hrs after ingestion of palmitic acid 1 C^{14} (mean±standard deviation

Number		Feces and intestine combined		Feces eliminated counts per 100 sec	Lipid from 1 ml of serum counts per 100 sec	Liver [illegible] counts per [illegible]
		Counts per 100 sec	% of dose ingested			
Ethionine rats	[illegible]	101,724±	56 2	393±	19±	[illegible] 08
		12,247		360	33	±
Saline	8	21 229±	11 7	2,2[illegible]9±	62±	[illegible]
		14 422		686	32	8
t value of difference		10 55		5 98	0 69	
P		0 001		0 001	None	0 00

and the acidified mixture was allowed to stand overnight Then the lipids were taken up in petroleum ether and washed with 50 % ethanol and with water

The serum lipids were extracted as described earlier (Karvinen *et al* 1964 Samples of the lipid were then plated on steel planchets and counted The counts were corrected for mass absorption

Results

In Table I the radioactivities remaining in the intestine of excreted in the feces are given It is seen that ethionine administration resulted in a significant reduction in the absorption of labeled palmitic acid from the intestine Radioactivity of the blood serum lipid was not significantly altered by the ethionine treatment, whereas that of the liver fat was tripled

Discussion

The present results indicate that the absorption of ingested palmitic acid 1 C^{14} from the intestine is slowed down in the ethionine treated female rat The experimental animals finished eating the ration containing the labeled fatty acid in 15 min, so it seems unlikely that a slower rate of intake of the labeled material by the ethionine animals would be responsible of the finding

The data on the radioactivity actually eliminated in the feces (Table I) suggest that the rate of propulsion of the gastro-intestinal contents in the ethionine-treated rats may be slightly slower than that of the saline controls Differing rates of propulsion could eventually lead to differing rates of absorption Since the process of fat absorption is completed already at the lower end of the small intestine and since some of the labeled fatty acid had already passed with the feces, it seems rather unlikely that a slower rate of propulsion in the gut of the ethionine rats would be the main cause of the slower absorption obtained in these animals

It is known that ethionine administration leads to acinar cell necrosis in the rat pancreas (Herman and Fitzgerald 1962) resulting in lowered pancreatic lipase

activities in the intestine (Libre and McFarland 1964) The pancreatic juice is rather important in the utilization of fat It has been shown that exclusion of pancreatic juice results in a decrease in the absorption of tripalmitin from rat's intestine, even though no significant change in the absorption of palmitic acid could be demonstrated in pancreatic duct-ligated rats (Karvinen *et al* 1957)

Supported by a grant from Sigrid Jusélius Foundation

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Acta physiol scand 1966. 68. 231—233

From the Department of Medical Chemistry University of Turku Finland

Collagen Components in the Consecutive Extracts of Rat Skin

By

E Heikkinen and E Kulonen

Received 30 March 1966

Abstract

Heikkinen, E and E Kulonen *Collagen components in the consecutive extracts of rat skin* Acta physiol scand 1966 *68* 231—233

The present results demonstrate that the various fractions of skin collagen are very different and that the solubility is a matter of definition The methods of various authors for the preparation of the soluble fractions of collagen vary greatly Standard procedures and a more defined terminology are urgently needed

The purpose of this note is to draw attention to the quite different component patterns in the collagen fractions which have been obtained with various solvents

That fraction of collagen which is soluble in dilute solutions of neutral salts is considered to be the youngest (Jackson 1957) It is assumed that collagen is continuously converted to less soluble and more mature forms by the formation of intra and intermolecular cross links and that in growing connective tissue there is a continuous spectrum of various aggregates of different ages (Jackson 1960 Heikkinen *et al* 1964a, b) By the present methods it is possible to test the properties of collagen solutions in the terms of the α β and γ (or x)-components (Nanto *et al* 1964) and consequently of the intra and intermolecular cross links

Various collagen fractions were obtained from skins of young rats (25—30 g) according to the flow sheet below where the amounts of each fraction are indicated Only 11 3 per cent of total collagen remained in the final insoluble fraction In Fig 1 the starch gel electrophoretic patterns of the components of denatured collagen fractions are presented Collagen which is extractable with dilute salt solution consists mainly of non linked α-components When the ionic strength of the solvent is increased additional collagen is dissolved and it contains also β-components with intramolecular cross links In acetic acid collagen swells and a great part of the residue dissolves This fraction is characterized by a dominance of β components and of larger aggregates, the x (or γ, δ) components Denatur

TABLE 1 Flow sheet of the extraction and purification of different collagen fractions from rat skin If not stated otherwise the manipulations were carried out at +4°C and the centrifugations with refrigerated equipment for 60 min at 35 000×g

---

Rat skin
was homogenized in 0 15 M NaCl (2 5 × wet weight) with Sorvall homogenizer for 2 min After 24 hr the mixture was centrifuged and the extraction was repeated four times

Residue
was extracted four times with 0 30 M NaCl and centrifuged

Combined supernatants
were filtered and dialyzed against 0 1 M citrate buffer, pH 3 6 Collagen was precipitated with sodium chloride (final concn 15%) collected by centrifugation and dissolved in 0 1 M acetic acid This procedure was repeated twice Collagen was finally precipitated by dialysis against several changes of 0 01 M disodium phosphate and water, collected by centrifugation dissolved in 0 1 M acetic acid and lyophilized The yield of 0 15 M NaCl-soluble fraction was 11 3% of total collagen

Residue
was extracted four times with 0 45 M NaCl and centrifuged

Combined supernatants
were handled as the 0 15 M supernatant The yield of 0 30-M NaCl-soluble fraction was 21 4% of total collagen

Residue
was extracted four times with 0 5 M acetic acid and centrifuged

Combined supernatants
were handled as the 0 15 M supernatant The yield of 0 45 M NaCl-soluble fraction was 18 2% of total collagen

Residue
was extracted once with 0 5 M acetic acid at +40 C for 30 min and centrifuged

Combined supernatants
were purified by precipitation with NaCl (final concn 10%) and subsequently by dialysis against several changes of 0 01 M disodium phosphate and water Collagen was collected by centrifugation dissolved in 0 5 M acetic acid and lyophilized The yield of acetic acid-soluble fraction was 29 4% of total collagen

Residue
was lyophilized The yield of the insoluble fraction was 11 3% of total collagen

Supernatant
was lyophilized The yield of warm acetic acid-extractable fraction was 8 4% of total collagen

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at rather low temperature (at + 40 C for 30 min) yields a further soluble fraction containing proportionally more of these larger fragments (cf Pikkarainen *et al* 1964) with intermolecular bonds *The last insoluble residue may* be solubilized to collagen components by digestion with pepsin Other work (Lampiaho *et al* 1965) shows that fractions of different metabolic ages can be demonstrated in conventional preparations of insoluble collagen

Supported by institutional grants from Sigrid Jusélius Foundation and U S Department of Agriculture Foreign Research and Technical Programs Division

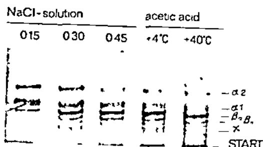


Fig 1 Starch gel electrophoretic patterns of the consecutive collagen fractions from rat skin The preparations are described in the flow sheet.

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Acta physiol. scand. 1966. 68. 234—245

From the Central Clinical Laboratory, Kommunehospitalet, Copenhagen, Denmark

Chloride and Hydrogen Ion Distribution between Human Red Cells and Plasma

By

JØRGEN FUNDER and JENS OTTO WIETH

Received 28 April 1966

Abstract

FUNDER, J and J O WIETH *Chloride and hydrogen ion distribution between human red cells and plasma* Acta physiol scand 1966 *68* 234—245

Distribution of Cl^- and H^+ between human red cells and plasma has been studied after variations of plasma pH (induced both by changing pCO_2 and changing bicarbonate concentration) The distribution ratio of chloride $Cl^-_{cell}/Cl^-_{plasma} = r(Cl^-)$ and of hydrogen ions $H^+_{plasma}/H^+_{cell} = r(H^+)$ showed the following relations to plasma pH

$$r(Cl^-) = 3\ 319 - (0\ 359\ pH_{plasma})$$

$$r(H^+) = 3\ 091 - (0\ 335\ pH_{plasma})$$

The conception that the distribution of Cl^- and H^+ is a Donnan distribution is supported by the distribution of Cl^- and H^+ found after addition of an impermeant negatively charged ion (citrate) to the extracellular phase The normally negative potential difference between cells and plasma may be inverted in the presence of citrate The effects on ion distribution seen when normal cells are suspended in isotonic sucrose or glucose can be explained as being caused by diffusion potentials which arise because of the much higher permeability of the red cell membrane towards anions than towards cations Calculation of the water shift to be expected as the osmotic consequence of the redistribution of diffusible anions between cells and plasma ($\Delta H_2O/\Delta pH$ 423 g H_2O kg RBC solids) was found to agree with the experimentally determined value of 401 g H_2O kg RBC solids

The conception that the distribution of hydrogen and chloride ions between cells and plasma is a Donnan equilibrium was introduced by the pioneering works of Warburg (1922) and of Van Slyke Wu and Mc Lean (1923) The validity of this theory has been generally agreed upon by many investigators (viz Dill, Edwards and Consolazio 1937, Fitzsimons and Sendroy 1961, and Bromberg et al 1965) However other works have questioned that the Donnan theory can always explain the distribution of hydrogen ions and rapidly diffusing anions between cells and plasma Peters, Tulin, Danowski and Hald (1947) found that when the distribution of chloride and combined CO_2 between cells and plasma of ox ... is altered, by varying the CO_2 tension ...

isoton ...

for ...

Bubnoff and Riecker (1961) found that hydrogen ion exchanges between cells and plasma after altering bicarbonate concentration took place very slowly, reaching equilibrium after one hour at 37° C Siggaard Andersen (1961) noted a considerable shift of hydrogen ions from plasma to cells lasting several hours, when blood containing sodium fluoride was stored anaerobically at 38° C He considered the possibility that the hydrogen ion shift observed was secondary to diffusion potentials created by passive diffusion of Na and K, (since K leaves the cells much faster than Na enters in the presence of fluoride) (Dunker and Passow 1950) However, it was also suggested that H^+ distribution in normal blood might be maintained by an active mechanism, extruding H^+ of the cells, and that the hydrogen ion shift observed might be a result of inhibiting the energy supply of such an H^+ transport with NaF No matter what is the cause of the transfer of hydrogen ions from plasma to cells in the presence of fluoride, this phenomenon questions the value of the results of Fitzsimons and Sendroy (1961) and of Bubnoff and Riecker (1961) Both these groups carried out experiments on cells in which glycolysis had been arrested by the addition of fluoride

The possibility that H^+ is transported actively into the red cell by a hypothetical carrier extruding Na^+ and accumulating K^+ and H^+ has been considered by Ussing (1960 p 78) Since reasonable doubt has been thrown on the passive distribution of H^+ between cells and plasma, this study was carried out on cells metabolizing at 38° C, since it might be argued that the separation of cells and plasma at 5—8° C as performed by Bromberg *et al* (1965) might inhibit an active transport mechanism as efficaciously as the employance of metabolic poisons It was thus found desirable to reinvestigate the distribution of Cl^- and H between cells and plasma with a technique that can eliminate the objections to previous investigations presented above In the present work chloride and hydrogen ion distribution of glycolyzing cells are examined in the plasma pH range 6 40 to 8 50 The variations found support the conception that a Donnan equilibrium exists across the red cell membrane with regard to chloride and hydrogen ions As a test of passive distribution of H and Cl^- it has been examined if the addition of an impermeant polyanion (citrate) to plasma leads to the predicted changes of ion distribution by neutralizing the effect of the non diffusible anions of the cell Finally it has been examined whether the water shift between cells and plasma which is observed after acute variations of plasma pH (Funder and Wieth 1966 a may be quantitatively explained as an osmotic consequence of a redistribution of diffusible ions secondary to a change in a Donnan potential between cells and plasma

Methods

Heparinized blood 10 i u per ml) was sampled from healthy donors Oxygenation and adjustment of CO_2 partial pressure was carried out by recirculating 50 100 ml of blood in a modified oxygenator unit of a heart lung machine (Frederiksen Ryge and Therkelsen 196? The following gas mixtures were employed for the adjustment of carbon dioxide partial pressures 1 5 6 % CO_2 94 4 % atmospheric air 2 80 % CO_2 20 % O_2 3 atmospheric air 0 03 % CO_2) Complete ox[illegible] of hemoglobin was ascertained by analysis (Zijlstra 1953 To study the influence of primary [illegible] of pCO_2 blood was exposed to the gas mixtures in the sequence 1 3, 2) No attempt w[illegible]

attain equilibration of the blood with the gas mixtures 2) and 3) Blood was drawn into a 10 ml syringe at random intervals, thereby obtaining a sequence of changes in pCO_2 The syringe was thermostated in a water bath at 38° C and after a 10 minutes temperature equilibration period 8 ml of blood were transferred to ...

iodinated albumin amounted to 1 1—1 5 per cent (w/w) by the centrifugation employed The isolated erythrocytes were immediately capped by a tight fitting PVC-cap Buffy coat was removed

RBC were immediately lyzed by freezing and thawing the tubes thrice in a dry ice-alcohol mixture After the last thawing the lyzed cells were immediately submitted to pH measurement at 38° C

0 14 M NaOH Addition of acid or base was performed in the following way After equilibration with gas mixture 1 (to ensure oxygenation 10 ml blood samples were spun at 1500 × g for 5 min 4 ml of plasma was withdrawn and acid or base added to the plasma Acid or base treated plasma was thereafter slowly added to the lightly packed cells After resuspending the red cells, blood was transferred to a 10 ml syringe and incubated at 38 C following the procedure described above The maximal additions of 0 14 M HCl or NaOH employed were 3 ml and 1 75 ml respectively This equals the addition of 42 meq acid and 24 5 meq base per litre of blood

Addition of Sodium Citrate Oxygenated blood was diluted with varying amounts of a solution of 0 05 M tertiary sodium citrate (0 15 eq Na per litre dissolved in a 2 5 % glucose solution 10 ml of citrate/blood mixtures were produced by mixing various amounts of blood and citrate solution (maximal amount of citrate solution 4 55 ml per 10 ml blood citrate mixture After 10 min incubation at 38° C the blood/citrate sample was treated further as described above

pH measurements were carried out at 38 C with micro electrode equipment (Electrode Radiometer E 5021 with pH-meter 25 SE The phosphate buffers (pH 6 84 and 7 38 at 38 C) used as standards of reference were in accordance with the NBS-scale (Bates 1954)

Chloride determinations in lyzed cells and plasma were carried out by electrometric titration with 0 01 M $AgNO_3$ (Titrator TTT1a Radiometer with silver electrode P 401 and calomel electrode (K601) with liquid junction of saturated K_2SO_4) As erythrocyte proteins are known to bind Ag

Terminology of Ion Distribution Ratios The relation between potential difference (E and the distribution of a freely diffusible ion in two phases separated by a semipermeable membrane is given by

$$\psi_o - \psi_i \qquad E = -\frac{RT}{zF} \ln \frac{(a)_i}{(a)_o} \text{ (Volt)}$$

where the subscripts i and o denote inside and outside phase respectively ψ is the electrical potential, R (8 314 joule °K⁻¹ mole⁻¹ is the gas constant T is the absolute temperature K z is the valency of the ion considered F Faradays constant (96493 coulomb eq⁻¹) and (a) designates the activity of the ion considered in the two phases indicated by the subscripts i and o A detailed

concentrations the tacit admission is made that the expression is not a correct ... difference, if the ratio of the activity coefficients of chloride ions in cells and plasma is not unity

TABLE I Hydrogen ion and chloride ion distribution between cells and ... the p... range 6 32—8 64 The regression lines of the dependency of distr... ratios ... pH are shown in Fig 1

Exp no	pH plasma	pH lyzed RBC	$r_{[H^+]}$	Cl plasma (meq kg H_2O)	Cl RBC (meq kg H_2O)	r_{Cl}	
pH changes induced by primary changes of pCO_2							
1	7 48	7 26	0 603	115	75 7	0 658	
	7 60	7 33	0 537	120	73 0	0 603	
	7 71	7 41	0 501	120	67.2	0 560	
	7 90	7 55	0 447	127	62 6	0 493	
	7 09	6 96	0 741	112	84 7	0 756	
	7 18	7 02	0 692	111	83 0	0 743	
	7 32	7 15	0 676	116	82 0	0 707	
	6 35	6 34	0 955	104	115	1 106	
2	7 36	7 16	0 631				
	7 80	7 46	0 457				
	7 35	7 14	0 617				
	6 74	6 65	0 813				
	6 52	6 46	0 871				
	6 44	6 40	0 912				
	6 32	6 32	1 000				
6	7 39	7 17	0 603	118	76 9	0 652	0 049
7	7 47	7 26	0 617	120	72 0	0 600	−0 017
8	7 36	7 15	0 617				
9	7 46	7 26	0 631				
10	7 33	7 17	0 692				
11	7 43	7 22	0 617				
12	7 43	7 21	0 603	113	74 6	0 660	0 057
pH changes induced by addition of HCl or NaOH							
3	6 73	6 67	0 871				
4	6 69	6 61	0 832	110	101	0 918	0 086
	6 50	6 46	0 912	111	109	0 982	0 070
	6 40	6 36	0 912	112	109	0 973	0 061
5	7 72	7 42	0 501	109	60 3	0 553	0 052
	7 83	7 47	0 437	109	53 7	0 493	0 056
	8 02	7 61	0 389	105	43 6	0 415	0 026
	8.26	7 76	0 316	105	38 0	0 362	0 046
6	6 42	6 39	0 933	117	113	0 966	0 033
	8 12	7 66	0 347	108	41 7	0 387	0 040
	8 50	7 90	0 251	104	29 6	0.285	0 034
7	6 63	6 59	0 912	129	126	0 977	0 065
	8 64	8 00	0 229				
9	7 52	7 28	0 575				
						Mean	0 052
						S D	0 031

In text, tables, and figures ion distribution ratios are indicated by r. The ratio of chloride in cells and plasma

Cl cell/Cl plasma is indicated as $r_{(Cl^-)}$,

correspondingly the ratio of hydrogen ion activities

H^+_{plasma}/H^+_{cell} is indicated as $r_{(H^+)}$

Results

1 *Distribution of H^+ and Cl^- as a function of plasma pH*

Data of chloride and hydrogen ion distribution between plasma and cell water found at various plasma pH values are given in Table I, and the regression lines of $r_{(Cl^-)}$ and of $r_{(H^+)}$ versus plasma pH are shown in Fig 1

The interdependence between hydrogen ion distribution and the pH of plasma was found to be expressed by the equation

$$r_{(H^+)} = 3.094\ (\pm 0.014) - 0.335\ (\pm 0.005)\ (pH_{plasma}) \quad (1)$$

The correlation coefficient of this regression line is -0.995 (± 0.015)

The regression line of the interdependence between $r_{(Cl^-)}$ and pH of plasma was similarly

$$r_{(Cl^-)} = 3.319\ (\pm 0.028) - 0.359\ (\pm 0.010)\ (pH_{plasma}) \quad (2)$$

The correlation coefficient of the regression line is -0.993 (± 0.026)

No differences were found in the ion distributions effected by changes of pCO_2, compared to the distributions found after addition of HCl or NaOH (viz Fig 1)

2 *Chloride and Hydrogen Ion Distribution in the Presence of Citrate in the External Medium*

It was felt that measurement of ion distributions under conditions, where the Donnan potential could be expected to be changed, or even inverted, would yield valuable information as to the legitimacy of evaluating the membrane potential of the red cell from the distribution of diffusible ions

It may be anticipated that the potential difference between cells and plasma can be diminished — or even inverted — if non-permeating anions are added to the

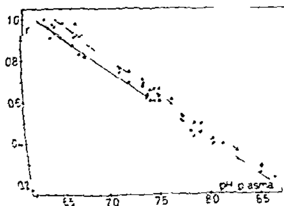


Fig 1 $r_{(Cl^-)}$ triangles and $r_{(H^+)}$ circles as functions of plasma pH Results indicated by open signatures were obtained after variation of pCO_2, results indicated by solid signatures after addition of HCl or NaOH The solid line represents the equation $r_{(H^+)} = 3.094 - 0.335\ pH_{plasma}$ The dashed line represents the equation $r_{(Cl^-)} = 3.319 - 0.359\ pH_{plasma}$

TABLE II Hydrogen ion and chloride ion distribution between cells and plasma under conditions where potential difference between cells and plasma is affected. Calculated potential differences agree within 2 mV in spite of the inversion of potential

Citrate conc. of ext. medium mM	pH ext. medium	pH lyzed RBC	$r_{(H^+)}$	Cl ext. medium meq/kg H_2O	Cl RBC meq/kg H_2O	$r_{(Cl^-)}$	Osmolality ext. medium	Calculated potential difference mV from $r_{(H^+)}$	from $r_{(Cl^-)}$
9.2	7.26	7.19	0.85	86.7	68.5	0.79	0.283	−4.4	−6.3
23.3	7.21	7.20	0.98	61.1	58.6	0.96	0.284	−0.6	−1.1
32.2	7.14	7.22	1.20	37.9	47.1	1.24	0.286	+4.9	+5.8
0[1]	7.12	7.16	1.10	36.8	37.6	1.02	0.290	+2.6	+0.5

[1] 5 ml blood + 5 ml 5% glucose

external medium. Since citrate is known to permeate the red cell membrane extremely slowly (if at all), the following experiment was designed.

Heparinized blood was diluted with a citrate solution (isotonic with plasma), containing 150 meq Na^+/litre and 2.5 % (w/v) glucose per litre to accomplish isotonicity with plasma. The results obtained are listed in Table II, which shows the pH of the external medium and lyzed cells, and the chloride concentration of plasma and cells after isolation carried out at 38° C. It appears from the results that the distribution of diffusible ions is affected in the predicted way by the presence of the non diffusible poly-anion in the plasma. Measurement of osmolality of the external medium (by determination of freezing point depression) showed only negligible changes. Potential differences — as calculated by inserting the values found for $r_{(Cl^-)}$ and for $r_{(H^+)}$ into the Nernst equation are shown in the far right columns. It is seen that the calculated potential differences are affected in the same direction and approximately to the same degree, whether they are estimated from hydrogen ion — or from chloride distribution. It further appears that the normal negative P.D. of the red cell under these conditions is inverted to positive values at a citrate concentration of 23 mM = 69 meq citrate per litre external medium.

The positive P.D. values obtained after adding citrate in sufficient concentration to the external medium is quite stable, as may be reasoned from the experiment shown in Fig. 2. This figure shows the recording of pH of the external medium of a blood citrate mixture with a citrate concentration of 32 mM. During an observation period of 1.5 hrs at 38° C pH of the medium only fell from 7.20 to 7.18. This fall of external pH may be due to continued metabolic activity of the RBC. However, a rise of external pH is to be expected if membrane potential was reverted during the experimental period due to penetration of citrate into the cells

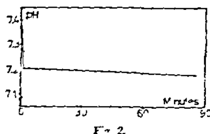


Fig. 2

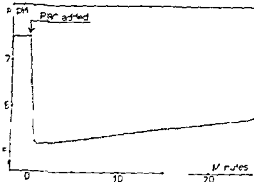


Fig. 3

Fig. 2. pH of the medium of a suspension of red cells incubated at 38 C. At zero time 5 ml of a citrate solution containing 50 mM citrate, 150 meq Na 1 and 2.5 w/v % glucose pH 7.40) is added to 5 ml of heparinized blood. Calculated citrate concentration of the extracellular phase 37 mM. After the initial pH change induced by mixing blood and citrate pH of the medium only showed a slight decreasing tendency. pH was measured continuously; readings were made with intervals of 0.5 to 5 min according to rate of pH change. Identical results were obtained in 5 experiments.

Fig. 3. pH of a solution of 5 % w/v glucose after the addition of 300 mg packed red cells to 10 ml glucose solution. The experiment was carried out at 22 C. pH was measured continuously; readings were made with intervals of 0.25 to 3 min according to rate of change. The figure is representative of a total of 20 expts.

3. *Chloride and Hydrogen Ion Distribution between Cells and 5 per cent Glucose*

Fig. 3 shows the effect on external pH caused by suspending 300 mg of packed RBC in 10 ml of a 5 % w/v glucose solution pH 7.20 Tp 22 C. The rapid initial fall of the environmental pH about 2 pH units — from pH 7.20 to 5.20 indicates a rapid redistribution of H and OH across the membrane induced by the diffusion of anions Cl and HCO_3 traversing the membrane at a rate which is initially 10^6 times faster than that of sodium and potassium (Passow 1964). The pH of the RBC employed in the experiment was 7.20.

It may be assumed that the pH of the erythrocyte fluid is virtually unaffected by a calculated liberation of 0.2 meq H/kg RBC, sufficient to lower pH of the unbuffered medium from 7.20—5.20. The maximal potential difference calculated by the Nernst equation from the maximal change of external pH is therefore 58 × 2 = 116 mV (cells positively charged relatively to medium). This potential may be regarded as a diffusion potential created by the tendency of the diffusible anions to distribute freely between cells and medium but being restrained by the much lower diffusibility of K (and Na). The potential is not stable because the cell under these abnormal conditions becomes leaky to cations. The escape of K from the cells (promoted by changed membrane permeability, concentration gradient, and potential gradient) will diminish the positive membrane potential. This is accompanied by a return of external pH towards initial values as seen in Fig. 3.

Measurements of ion distributions by the methods employed in this study are not feasible in a rapidly changing situation as the one described above. However, a

related — albeit less marked and considerably slower — phenomenon may be induced by diluting a sample of heparinized blood with an isotonic solution of non electrolyte The values given in the lowest line of Table II, show the results of measuring chloride and hydrogen ion distribution in medium and erythrocytes isolated at 38° C, after diluting a 5 ml blood sample with 5 ml of 5 % glucose As in the case after addition of citrate to the medium fair agreement is found between the distribution of chloride and hydrogen ions, and the values of potential differences calculated from the distribution ratios

Discussion

The almost parallel course of the regression lines of $r_{(Cl^-)}$ and of $r_{(H^+)}$ versus plasma pH seen in Fig 1 suggests a simple relationship between chloride and hydrogen ion distribution in cells and plasma as would be expected for the distribution of freely diffusible ions in a Donnan system Contrasting the findings of Peters *et al* (1947) we found that the distribution ratios of Cl^- and H^+ were independent of the way in which pH changes were produced — by altering pCO_2 or by adding acid or base

A significant difference is found in the absolute values of $r_{(Cl^-)}$ and of $r_{(H^+)}$ (*viz* Table I) At identical plasma pH chloride distribution ratio was found to exceed $r_{(H^+)}$ by a mean value of 0 052 (S D 0 031, SEM 0 007, $p < 0\ 001$)

It is not possible to determine the cause of this systematical difference A difference in the activity coefficients of chloride in cells and plasma, protein binding of chloride trapping of extracellular chloride between the packed cells, and errors arising from liquid junction potentials in the pH measurements of lyzed cells are all probable causes

The Effect of Citrate Chloride concentration ratio and hydrogen activity ratio were found to be affected to the same degree by the addition of citrate to the external medium (Table II) This supports the conception that both distribution ratios reflect changes of the potential difference between cells and plasma However it must be admitted that it is not possible to decide whether $r_{(Cl^-)}$ or $r_{(H^+)}$ gives a fully correct clue to the calculation of membrane potential Values calculated by the two ratios differ by approximately 2 mV in the pH range considered Direct recording of the RBC membrane potential has not yet been possible so we have no better estimates than those calculated from $r_{(Cl^-)}$ and $r_{(H^+)}$

The Effects of "Low Electrolyte Media The term low electrolyte media was employed by Wilbrandt and Schatzmann (1960) to characterize a number of media isotonic to plasma in which RBC exhibits a series of peculiar membrane phenomena due to the fact that electrolytes have been largely or completely replaced by slowly permeating non electrolytes as sucrose lactose mannitol or in the case of human RBC — high concentrations of glucose For a detailed review of the effects on ion movements membrane permeabilities and membrane potential the reader is referred to the above cited work Here shall only be stated that the dysequilibrium caused by the non-electrolyte medium can be appreciated from the pH changes o the medium seen in Fig 3

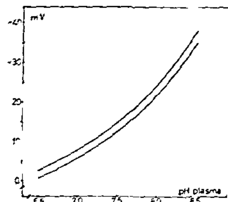


Fig 4

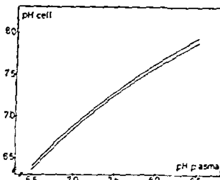


Fig 5

Fig 4 Potential difference between red cells and plasma (calculated by equation 3) as a function of plasma pH The upper line of the diagram was calculated from $r_{(H^+)}$ the lower line from $r_{(Cl^-)}$

Fig 5 Cellular pH (calculated from equation 4 as a function of plasma pH The upper line of the diagram was calculated from $r_{(Cl^-)}$ the lower line from $r_{(H^+)}$

Peters *et al* 1947) found that dilution of blood with isotonic succrose caused the distribution of H and Cl to deviate from a Donnan equilibrium The experiment of Peters *et al* has been repeated by us lowest line of Table II) We found that distributions of H and Cl were affected in the expected way and to the same degree when blood was diluted with isotonic glucose

Membrane Potential and Intracellular pH If the activities of chloride and hydrogen ions could be determined the potential difference (E between plasma and cells and the hydrogen ion activity of RBC could be calculated In the following we have attempted to estimate the magnitude of the membrane potential and the intracellular pH of red cells by transposing the Nernst equation inserting the values of $r_{(Cl^-)}$ and of $r_{(H^+)}$ given by equations 1 and 2 However, it must be born in mind that the ion distribution ratio was calculated from determinations of *chloride concentrations* Further pH measurements on lyzed cells may be biased by liquid junction potentials The following calculations must consequently be taken with reservations

$$E = 61\ 7 \log r \quad mV \text{ at } 38\ C \qquad (3)$$

Fig 4 shows the values of E calculated from $r_{(Cl^-)}$ and from $r_{(H^+)}$ The membrane potential at a plasma pH of 7 40 is thus found to be a 11 mV as calculated from chloride distribution , and b 13 mV when calculated from hydrogen ion distribution)

Intracellular pH was similarly evaluated from the relation

$$pH_{cell} = pH \text{ plasma} + \log r \qquad (4)$$

Fig 5 shows the values of cell pH at various plasma pH levels The pH of red cells at a plasma pH of 7 40 is thus found to be 7 19 by direct measurement of pH on lyzed cells, and 7.22 when calculated from the chloride distribution

Water Shifts between Cells and Medium as a Consequence of Donnan Distribution of the Diffusible Anions

The diffusion rates of the anions Cl^- and HCO_3^- through the red cell membrane exceed those of the cations K^+ and Na^+ by a factor of 10^6 It might therefore be anticipated that the volume changes of red cells caused by acute changes of extra cellular pH are due to water movements following the redistribution of diffusible anions

The calculations below were based on the following assumptions

1) Water is present in osmotic equilibrium on the two sides of the RBC-membrane Movement of an osmotically active particle from one phase to another causes the concomitant transfer of an equivalent amount of water At an osmolality of 0 3 osmoles/kg H_2O this equivalent amount is $\frac{1000}{300} = 3\ 33$ g H_2O per milliosmole

2) The chloride distribution ratio (eq 2) is representative of the distribution of all diffusible monovalent anions

If the concentration of extracellular diffusible anions is kept constant (134 meq A^-/kg H_2O), the concentration of A^- in the water phase of the RBC can accordingly be calculated from $r_{(Cl)}$

A^- (pH 7 0) $= 134 \times 0\ 806 = 108\ 0$ meq/kg H_2O, and

A^- (pH 8 0) $= 134 \times 0\ 447 = 59\ 9$ meq/kg H_2O

If we consider 1 kg RBC at a plasma pH of 7 0 the following composition may be calculated from the data of Funder and Wieth (1966 a, b)

plasma pH 7,0, RBC 1000 g, cell water 681 g, cell solids 319 g, A 108 0 meq/kg RBC H_2O, 73 5 meq/kg RBC Cell water related to cell solids 2132 g H_2O kg solids

Since, according to assumption 1), one meq A^- moves together with 3 33 g H_2O, the loss of water and of anions that will establish a distribution ratio of 0 447 can be calculated This is found to be the case in the above example after a loss of 40 8 meq A^- and 134 6 g H_2O respectively The original one kilogram of RBC considered at pH 7 0 accordingly shrinks by 134 6 g by an increase of extracellular pH from 7 0 to 8 0 We therefore have

plasma pH 8 0 RBC 865 g, cell water 546 g cell solids 319 g A^- 59 9 meq kg RBC H_2O, 32 7 meq/865 g RBC Cell water related to cell solids 1710 g H_2O kg solids

It is thus found that the amount of water kg RBC solids is reduced from 2132 g at pH 7 0 to 1710 g at pH 8 0 This calculated reduction of 422 g H_2O kg RBC solids should be compared to the experimental finding of 404 g H O kg RBC solids (S E 23, n = 15 Funder and Wieth 1966 a This agreement justifies the conception that the water shift caused by acute change of extracellular pH is almost solely due to the shift of anions secondary to the establishment of a new Donnan equilibrium

Comparison to Previous Investigations The literature up to 1965 has recently been reviewed in the original study of Bromberg, Theodore Robin and Jensen

TABLE III Chloride distribution between cells and plasma as a function of plasma pH Values from four materials In the outer right columns are shown chloride distribution ratio at a plasma pH of 7 40 and the values of cellular pH and potential difference between cells and plasma (P D) at plasma pH 7 40 as found by inserting the values of $r_{(Cl^-)}$ into eq ns 3 and 4

Author	Relation of $r_{(Cl^-)}$ to plasma pH	$r_{(Cl^-)}$ at pH_{plasma} 7 40	pH cell calc from $r_{(Cl^-)}$	P D (at 38°C) mV
Dill et al (1937)	$(-0\ 290 \times pH_{plasma}) + 2\ 820$	0 674	7 229	− 10 8
Fitzsimons & Sendroy (1961)	$(-0\ 344 \times pH_{plasma}) + 3\ 208$	0 662	7 221	− 11 1
Bromberg et al (1965)	$(-0\ 311 \times pH_{plasma}) + 2\ 928$	0 627	7 197	− 12 5
Present material	$(-0\ 359 \times pH_{plasma}) + 3\ 319$	0 662	7 221	− 11 0

TABLE IV Hydrogen ion distribution between cells and plasma as a function of plasma pH Values from three materials In the outer right columns are shown hydrogen ion distribution ratio at a plasma pH of 7 40 and the corresponding values of cellular pH and potential difference between cells and plasma (P D) as found by inserting the values of $r_{(H^+)}$ into eq ns 3 and 4

Author	Relation of $r_{(H^+)}$ to plasma pH	$r_{(H^+)}$ at pH_{plasma} 7 40	pH cell at pH_{plasma} 7 40	P D (at 38 C) mV
Fitzsimons & Sendroy (1961)	$(-0\ 440 \times pH_{plasma}) - 3\ 883$	0 627	7 197	12 5
Bromberg et al (1965)	$(-0\ 338 \times pH_{plasma}) - 3\ 062$	0 561	7 149	− 15 5
Present material	$(-0\ 335 \times pH_{plasma}) - 3\ 094$	0 615	7 189	13 0

so it only seems necessary to relate their work to the present, and add a few comments to the older literature on the item

Table III shows the regression equations of $r_{(Cl^-)}$ versus pH of plasma as found by four groups It is seen that the results agree fairly well

The hydrogen ion distribution as a function of external pH as found by Bromberg and associates (1965), by Fitzsimons and Sendroy (1961) and by us is shown in Table IV The slightly lower values of $r_{(Cl^-)}$ and of $r_{(H^+)}$ as found by Bromberg *et al* (1965) may be explained by the fact that these authors separated cells from plasma at 5—8° C As the Δ pH/Δ T coefficient is steeper for cells than for plasma, the pH measured on lyzed cells after rewarming to 38 must be expected to be lower, and the pH measured on plasma after separation in the cold and subsequent rewarming correspondingly higher, than the values obtained by separating cells and plasma at the temperature of pH measurement

Other estimates of intracellular pH in red cells Attempts to apply the determination of the distribution between cells and plasma of the weak acid 5 5 dimethyl 2 4, oxazolidinedione (DMO) as an indirect measure of H distribution across the red

cell membrane have not been successful Thomason (1963) found from studies of DMO distribution that RBC-pH at a plasma pH of 7 35 averaged 7 29, wheras Bromberg *et al* also employing DMO found pH values, which were markedly lower than the values of pH measured directly on the lyzed cells At a plasma pH of 7 40 the pH of cells was determined to be 7 09 from the DMO distribution, as contrasted by the value 7 15 found by direct pH measurement on lyzed cells, and 7 20 as evaluated from $r_{(Cl^-)}$ Bromberg and associates could not offer any explanation of the large discrepancy between the results obtained by Thomason and by themselves by means of DMO However, these results urge to reluctance in accepting DMO distribution ratios as a true measure of intracellular pH values

This study was supported by a grant from Fonden til lægevidenskabens fremme The results were presented at the IX Scandinavian Congress of Clinical Chemistry Sandefjord 1963 The valued technical assistance of Mrs Karen Meibom is gratefully acknowledged

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Acta physiol. scand. 1966. 68. 246—254
From the Institute for Medical Biochemistry University of Oslo, Norway

The Action of Insulin on Glycogen Synthesis in Rat Diaphragm

A comparative study of in vitro and in vivo effects

By

ODDMUND SØVIK

Received 29 April 1966

Abstract

SØVIK, O *The action of insulin on glycogen synthesis in rat diaphragm A comparative study of in vitro and in vivo effects* Acta physiol scand 1966 *68* 246—254

Insulin administered by intraperitoneal injection in doses which did not significantly alter the

regulation of muscle glycogen synthesis *in vivo*

In 1960 Villar Palasi and Larner reported an increased activity of glycogen synthetase (UDP-glucose α-1,4-glucan α-4-glucosyltransferase, EC 2 4 1 11) in rat diaphragm muscle after insulin treatment *in vitro* Glycogen synthetase has been shown to occur in two forms, one active in the absence of glucose-6-P (independent form, synthetase I), and another which depends upon this ester for its activity (dependent form, synthetase D) (Rosell-Perez *et al* 1962, Traut and Lipmann 1963)

In the cut diaphragm, originally studied by Villar-Palasí and Larner, insulin was shown to cause an increased activity of the independent form of the enzyme, without changing the activity measured in the presence of added glucose-6-P This insulin effect was observed in the absence of glucose or any other substrate in the incubation medium (Villar-Palasí and Larner 1961) and no concomitant increase in the tissue levels of glucose-6-P could be detected (Larner *et al* 1964 It was therefore suggested that insulin stimulated glycogen synthetase D → I conversion This offered an explanation of the so-called "directive" effect of insulin on glycogen synthesis (Beloff-

Chain *et al* 1956, Norman *et al* 1959, Larner *et al* 1960), and suggested an ability of insulin to regulate enzyme activities

The present investigation was undertaken to study the physiological significance of the glycogen synthetase activating effect of insulin The main question was whether the effect of insulin on the conversion of synthetase D to synthetase I in diaphragm could be reproduced *in vivo* Rafaelsen (1963) observed that intraperitoneal injection of small quantities of insulin into rats produced a marked stimulation of glycogen synthesis in the diaphragm without any significant effect on blood glucose With this technique the effect of insulin *in vivo* could be studied under conditions where counter actions from the organism as a whole were minimized

The results demonstrate that the increased activity of synthetase I in the diaphragm found after insulin *in vitro* was not observed after intraperitoneal injection of insulin However, the insulin induced glycogen synthesis, determined by the incorporation of ^{14}C-glucose, was greater under *in vivo* conditions

Methods

In vivo experiments Rats of the local strain weighing 100—160 g and fasted for 18—24 hrs received an intraperitoneal injection of 1 ml 0 9 % NaCl with or without insulin The injection was made with a morphin cannula to the right of the umbilicus The standard dose of insulin injected was



ion and analytical procedures

Analytical techniques Glycogen synthetase was assayed essentially according to Leloir *et al* (1959 Details of the assay have been described elsewhere Søvik *et al* 1966 The results are expressed as μmoles UDP formed per hour per g wet muscle tissue or per mg muscle protein The synthetase I activi
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min and the supernatant neutralized with 5 M potassium carbonate using methyl orange as an indicator Glucose 6-P was estimated from the increase in absorbance at 340 mμ upon addition of 10 μg
8 4
was
Carr
N HClO₄ After centrifugation glucose
method of Huggett and Nixon (1957)

Materials Insulin 10 crystallized
(specific activity 100 mC mmole) w

TABLE I Acid base parameters in arterial plasma and in brain tissue of rats which were either uninjected or injected with 2 or 3 doses of 50 mg Diamox per kg every second hour T_{CO} is the total CO_2 content of the brain P_{tCO_2} the mean tissue CO_2 tension The standard bicarbonate content of the tissue phase was obtained by displacing the experimental points along the buffer curve of the tissue to a CO_2 tension of 40 mm Hg (see Fig)

	Arterial plasma			Brain tissue			
Rats	pH	P_{CO_2} mm Hg	Actual HCO_3 meq/l	T_{CO_2} mmoles/kg	P_{tCO_2} mmHg	Actual HCO_3 meq/l	Stand HCO_3 meq/l
Uninjected controls	7 43 ±0 01	40 2 ±1 9	24 8 ±1 1	14 1 ±0 3	46 5 ±1 7	16 1 ±0 3	15 3 ±0 1
Diamox 100 mg/kg (3 hrs)	7 26 ±0 02	42 0 ±1 9	17 3 ±0 3	15 8 ±0 3	48 2 ±1 8	18 6 ±0 4	17 ? ±0 4
Diamox 150 mg/kg (6 hrs)	7 22 ±0 01	46 0 ±3 1	17 3 ±1 0	15 6 ±0 3	51 9 ±2 9	17 6 ±0 3	16 2 ±0 3

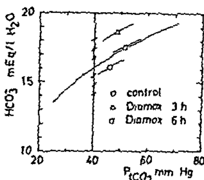


Fig Relation between the mean bicarbonate content and the tissue CO_2 tension in uninjected rats and in rats injected with 100 mg/kg (3 h) or 150 mg/kg (6 h) of Diamox The short bars on the 40 mm Hg line denote base excess units

CL 13,850 had no significant effect on the tissue bicarbonate content Control experiments further showed that there was no increase in the CSF bicarbonate concentration after acetazolamide at these equilibration times This indicates that the observed increase in the tissue bicarbonate represented an increase in the intracellular bicarbonate concentration The mechanisms behind this increase may be related to the action of the carbonic anhydrase in the tissue cells These mechanism are being further investigated

Supported by the Swedish Medical Research Council (Project no 14X 263 02 and by contract N62558-4988 between the Office of Naval Research Department of the U S Navy and the University of Lund Sweden

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Methods

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In deca phragms w 20 ml med perf med O The m u um ed 7 2 7 4 separa e the t ssu a this tion anc ... ucal procedures

Anal ical techn que Glycogen synthetase was assayed essent ally accord ng to Lelo r *et al* (1959 Deta s of he assay ha e been described elsewhere (So k *et al* 1966) The results are expressed as μmoles UDP formed per hour per g wet muscle t ssue or per mg muscle prote n The s nthetase I activ y s a so calculated as a percentage of the total activ ty (the act v ty in the presence of excess g u ose-6-P In some exper m ... he ng of

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$HClO_4$ After centr fuga on glucose was determ ned n the supernatant by the glucose ox dase method of Hugg tt and N xon (1957) Prote n was determ ned as described by Lowry *et al* (1951)

M erials Insul n 10 crystall zed was obta ned from No o Industr A S UDP C-glucose (spec fic a ty > 100 mC mmole) was purchased for New England Nuclear Corporation UDP

Blood samples drawn form the ta l vessels were deprote nized w th 0 33

TABLE I Effect of insulin on glycogen synthesis in the isolated intact diaphragm and in diaphragm *in situ*. The isolated diaphragms were incubated as described under "Methods" In the *in vivo* experiments the animals received intraperitoneal injections of 2 μC ^{14}C-glucose with or without insulin In some experiments samples for determination of blood glucose were taken 60—90 min after the injection The animals were killed after 150 min Blood glucose, amount of glycogen and labelling of glycogen by ^{14}C-glucose were determined as described under "Analytical techniques" The number of observations is given in parentheses

Experimental conditions	Glycogen		Blood glucose
	μg 100 mg wet tissue[1]	c p m /100 mg wet tissue[1]	mg 100 ml
Diaphragm *in situ*			
Control	264 ± 32 (6)	285 ± 103 (6)	114 3 ± 4 1 (5)
Insulin	1 188 ± 90 (6)	11 838 ± 2 530 (6)	109 5 ± 2 6 (6)
Isolated diaphragm			
Control	166 ± 19 (9)	1 417 ± 228 (9)	
Insulin	165 ± 18 (9)	8 533 ± 1 320 (9)	

[1] ± Standard error of the mean.

ucose glycogen, glucose-6-P phosphoenolpyruvate pyruvate kinase and NADP were purchased n Sigma Chemical Corporation. Glycogen was passed over a column of Norit before use Glucose-6-P dehydrogenase was obtained from C F Boehringer & Soehne Chemical and biochemical reagents not mentioned above were of standard analytical grade

Statistical methods Each observation is based upon analyses in duplicate of muscle tissue or blood from one animal The significance of a difference between two groups of animals was calculated by "Student's t test P values were obtained from Croxton (1959

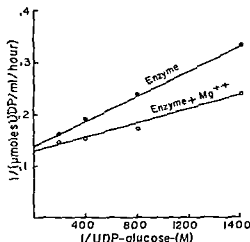
Results

Effect of insulin on glycogen synthesis Table I shows the effect of insulin on glycogen synthesis in the isolated diaphragm compared with the diaphragm *in situ* *In vitro* the labelling of glycogen by ^{14}C-glucose was increased 6-fold by insulin 0 1 U ml This insulin effect was not essentially increased by changing the incubation time by adding substrate to the medium, or by increasing the hormone dose Intraperitoneal injection of insulin 10 mU 100 g had a more marked effect on glycogen synthesis Measured 150 min after insulin injection the [illegible] lling of glycogen in diaphragms from insulin trea[illegible] n in the controls The [illegible] As demonstrated [illegible] the diaphragm levelled off 120 min after ins[illegible] The blood glucose of insulin treated animals tested 60—90 min after the injection, did not differ significantly from controls Table I

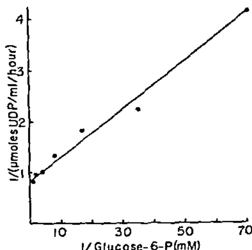
Characterization of glycogen synthetase When a crude enzyme extract containing about 90 % synthetase I was studied, the apparent K_m for UDP-glucose was 9×10^{-4} M (Fig 1) This is similar to values previously reported for the rat muscle enzyme (Rosell Perez *et al* 1962) Mg lowered the K_m-value without affecting V_{max}

EDTA pH 7 8 The homogenate was centri-

to the standard procedure, except for varying the concentration of UDP glucose In some experiments Mg^{++} was added



for 20 min and the supernatant assayed for glycogen synthetase according to the standard procedure, except for varying the concentration of glucose 6-P



The effect of varying the concentration of glucose 6-P was also studied (Fig 2) The apparent K_a for the activation of synthetase D by glucose-6-P was 5 5 × 10⁻⁴ M, a value close to that reported by Leloir *et al* (1959

Effect of insulin on glycogen synthetase The activity of glycogen synthetase was studied in extracts from diaphragms incubated with insulin Table II) Insulin caused a rise in synthetase I from 9 4 to 17 9 %, while the total enzyme activity remained unchanged Table II also shows activities of glycogen synthetase in extracts from diaphragms after insulin *in vivo* In contrast to the results obtained with diaphragm *in vitro*, insulin had no effect on the amount of synthetase I The percentage of synthetase I was almost unchanged throughout 4 hrs after insulin injection (I
The same result was obtained when UDP-^{14}C-glucose was used for gly

TABLE II Effect of insulin on glycogen synthetase in the isolated intact diaphragm and in diaphragm *in situ* The experimental conditions were similar to those described in the legend of Table I except that ^{14}C-glucose was omitted Glycogen synthetase was assayed as described under Analytical techniques The number of observations is given in parentheses

Experimental conditions	Glycogen synthetase		Percentage of I form
	No glucose 6 P	Glucose 6 P added	
	μmoles UDP/g wet tissue/hr¹		
Isolated diaphragm			
Control	*13 1 ± 2 4 (9)	140 0 ± 7 4 (9)	9 4
Insulin	*25 2 ± 3 4 (9)	142 0 ± 6 4 (9)	17 9
Diaphragm in situ			
Control	18 3 ± 2 1 (6)	202 0 ± 14 3 (6)	9 0
Insulin	15 6 ± 1 0 (6)	177 5 ± 15 3 (6)	8 7
	μmoles UDP mg protein hr¹		
Control	0 99 ± 0 29 (4)	8 78 ± 0 21 (4)	
Insulin	0 97 ± 0 43 (4)	8 52 ± 0 56 (4)	

¹ ± Standard error of the mean
* p 0 01

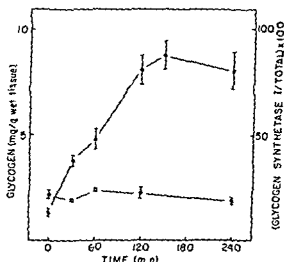


Fig 3 *Percentage of synthetase I and amount of glycogen in diaphragms after intraperitoneal injection of insulin* Insulin was administered according to the standard procedure and the animals were killed after the time periods indicated Glycogen and glycogen synthetase were assayed as described under analytical techniques One hemidiaphragm was used for glycogen while the other was used for enzyme determination Each value for glycogen represents the mean of 5—10 and each value for glycogen synthetase the mean of > 6 observations The length of the vertical bars shows two standard errors of the mean
Glycogen ●——●
synthetase I ○——

thetase determination In some of the *in vivo* experiments insulin seemed to suppress the enzyme activities Table II This effect was not present when the muscle protein, rather than the wet weight of the muscle was used as a reference for the enzyme activity The reason for the effect is supposedly an increased tissue weight after insulin injection due to glycogen accumulation

TABLE III Amount of glycogen and synthetase I in the 105 000 × g particulate fraction of diaphragm muscle homogenate after insulin *in vivo* In each experiment insulin was administered to 5 rats according to the standard procedure while 5 rats received saline The animals were killed after 150 min and hemidiaphragms were cut out and immediately frozen in liquid O_2 The following procedure was carried out in the cold room (2° C) The experimental as well as the control diaphragms were pooled powdered and extracted (1 20 w/v) with 0 05 M tris — 0 005 M EDTA pH 7 8 in a Potter Elvehjem homogenizer The homogenates were centrifuged at 5 000 × g for 10 min The sediment was discarded while 12 ml of the supernatant was centrifuged in a Spinco ultracentrifuge (Model L) for 5 hrs The small sediment was homogenized with 3 ml of the original buffer and analyzed for glycogen and enzyme as described under "Analytical techniques" The number of observations is given in parentheses

Experimental conditions	Glycogen	Glycogen synthetase
	μg/ml[1]	percentage of I form[1]
Control	25 7 ± 12 8 (3)	18 7 ± 1 5 (3
Insulin	214 4 ± 50 9 (3)	18 4 ± 0 7 (3)

[1] ± Standard error of the mean

TABLE IV Effect of insulin on glucose 6-P in the diaphragm The experimental conditions were as described in the legend of Table I except for the omission of ^{14}C-glucose Glucose-6-P was determined as described under Analytical techniques The number of observations is given in parentheses

Experimental conditions	Glucose 6-P
	μmoles g wet tissue
Diaphragm *in situ*	
Control	[1]0 76 ± 0 06 (9)
Insulin	[2]1 15 ± 0 11 (9)
Isolated diaphragm	
Control	0 10 ± 0 04 (4)
Insulin	0 09 ± 0 04 (4)

[1] ± Standard error of the mean
[2] p < 0 01

The activity of glycogen synthetase in the 105 000 × g particulate fraction of the diaphragm muscle homogenate was studied Table III In this fraction the specific activity of the enzyme was 8—9 fold higher than in the crude muscle extract While insulin *in vivo* caused a 10-fold increase in the amount of glycogen in this fraction, the percentage of synthetase I remained unchanged

The possibility was tested that insulin injection and subsequent incubation of the diaphragm might reveal a hormone effect on synthetase I Rats were

min after intraperitoneal injection of insulin Intact diaphragms were prepared and incubated 60 min at 37° C in Krebs Ringer phosphate buffer with glucose (2 mg/ml) However, the amount of synthetase I was the same in diaphragms from insulin-treated and control animals

Effect of insulin on glucose 6 P In diaphragms *in vitro* the glucose 6 P levels were low, and there was no effect of adding insulin to the incubation medium (Table IV) The basic levels of glucose 6-P determined in diaphragms frozen immediately after excision were considerably higher, and close to the apparent K_a for synthetase D (Fig 2) The amount of glucose 6-P was compared in diaphragms removed 150 min after intraperitoneal injection of saline with and without insulin After insulin the amount of glucose 6 P was substantially increased, and reached values well above the apparent K_a for synthetase D

Discussion

The increase in glycogen synthetase I in rat diaphragm after incubation with insulin is well documented (Villar Palasí and Larner 1960, Danforth 1965), and is also demonstrated in the present work The glycogen synthetase D ⇄ I interconversion probably involves phosphorylation by a specific kinase and dephosphorylation by a specific phosphatase (Friedman and Larner 1963) Whether insulin inhibits the kinase or stimulates the phosphatase system is unknown

In contrast to the *in vitro* results, intraperitoneal injection of insulin did not cause any increase of synthetase I in the diaphragm There are two possible explanations for the striking discrepancy between the *in vivo* and the *in vitro* results Firstly, the increase of synthetase I might be reversed during preparation and handling of the tissue Secondly, the effect of insulin on synthetase I thought present *in vitro*, might not occur in the intact rat organism The removal of an organ or a tissue frequently causes adrenalin secretion, muscle convulsions or anoxia Adrenalin will suppress the total glycogen synthetase activity (Belocopitow 1961), as well as the amount of the I form (Larner *et al* 1963, Danforth 1965) Muscle convulsions Danforth 1965) or anoxia (Sovik *et al* 1966) will increase synthetase I activity For the following reasons these factors were supposed to be of minor importance 1 The percentage of synthetase I was found to be the same in diaphragms prepared after decapitation of the animals and when prepared under anesthesia 2 A similar percentage of synthetase I was observed in diaphragms incubated under basal aerobic conditions 3 The synthetase D ⇄ I interconversion in muscle is supposed to operate in minutes rather than seconds Danforth 1965 In the present investigation the diaphragm was always frozen within one minute after the animals were killed

The diaphragm *in vivo* is performing rhythmical mechanical work whereas the isolated diaphragm is a resting muscle, unless stimulated by the isolated *nervus phrenicus* However, information on the effect of mechanical work on glycogen synthetase activity is lacking, and it is at present difficult to explain the observed discrepancy between the *in vivo* and the *in vitro* results in terms of difference in mechanical work

The effect of intraperitoneal injected insulin on glycogen deposition in the diaphragm is dramatic It occurs with doses of insulin too small to alter the concentration of blood glucose The observation that synthetase I remains unchanged suggests that insulin *in vivo* can markedly enhance glycogen synthesis in this muscle without causing any synthetase D → I conversion

It is debatable whether, in the absence of an insulin effect on synthetase I, the effect of insulin on glucose transport (Park *et al* 1955, Park and Johnson 1955) could account for the increased glycogenesis It has been claimed (Norman *et al* 1959) that when glucose entry into diaphragm is increased by raising the glucose concentration in the incubation medium, no stimulation of glycogen synthesis comparable to that induced by insulin occur This conclusion was based upon experiments *in vitro*, and whether it is valid under *in vivo* conditions is not known

Glucose-6 P might play an important role in the regulation of muscle glycogenesis *in vivo* After intraperitoneal injection of insulin the concentration of glucose 6 P in diaphragm was raised by 50 per cent, and reached a value above the apparent K_a for synthetase D Although formation of glucose 6 P from glycogen during preparation of the tissue might have contributed somewhat to the glucose 6 P levels observed, this would not change the absolute difference observed with and without insulin It is likely that the change in glucose 6 P levels in response to insulin caused a substantial increase in the total glycogen synthetase activity

The author would like to thank Professor O Walaas for his continuous interest in the present investigation This work was supported by grants from Nordic Insulin Fund and from The Norwegian Council for Science and The Humanities The technical assistance of Mrs A Adler and Mrs A Eggesvik is acknowledged

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TABLE I Acid base parameters in arterial plasma and in brain tissue of rats which were either uninjected or injected with 2 or 3 doses of 50 mg Diamox per kg every second hour T_{CO_2} is the total CO_2 content of the brain P_{tCO_2} the mean tissue CO_2 *tension* *The standard* bicarbonate content of the tissue phase was obtained by displacing the experimental points along the buffer curve of the tissue to a CO_2 tension of 40 mm Hg (see Fig)

	Arterial plasma			*Brain tissue*			
Rats	pH	P_{CO_2} mmHg	Actual HCO_3^- meq/l	T_{CO_2} mmoles/kg	P_{tCO_2} mmHg	Actual HCO_3^- meq/l	Stand HCO_3^- meq/l
Uninjected controls	7 43 ±0 01	40 2 ±1 9	24 8 ±1 1	14 1 ±0 3	46 5 ±1 7	16 1 ±0 3	15 3 ±0 1
Diamox 100 mg/kg (3 hrs)	7 26 ±0 02	42 0 ±1 9	17 3 ±0 3	15 8 ±0 3	48 2 ±1 8	18 6 ±0 4	17 7 ±0 4
Diamox 150 mg/kg (6 hrs)	7 22 ±0 01	46 0 ±3 1	17 3 ±1 0	15 6 ±0 3	51 9 ±2 9	17 6 ±0 3	16 2 ±0 3

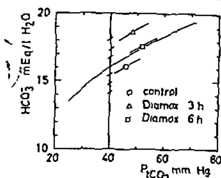


Fig Relation between the mean bicarbonate content and the tissue CO_2 tension in uninjected rats and in rats injected with *100 mg/kg (3 h)* or *150 mg/kg (6 h)* of Diamox The short bars on the 40 mm Hg line denote base excess units

CL 13,850 had no significant effect on the tissue bicarbonate content Control experiments further showed that there was no increase in the CSF bicarbonate concentration after acetazolamide at these equilibration times This indicates that *the observed increase in the tissue bicarbonate represented* an *increase in the* intra cellular bicarbonate concentration The mechanisms behind this increase may be related to the action of the carbonic anhydrase in the tissue cells These mechanisms are being further investigated

Supported by the Swedish Medical Research Council (Project no 14X 263 02) and by contract N62558 4288 between the Office of Naval Research *Department of the U S Navy and the University* of Lund Sweden

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Inhibitory Action of Adrenergic Blocking Agents on Catecholamine Release and Uptake in Isolated Nerve Granules

By

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Received 28 March 1966

Abstract

Euler, U. S. v. and F. Lishajko, *Inhibitory action of adrenergic blocking agents on catecholamine release and uptake in isolated nerve granules.* Acta physiol. scand. 1966. 68. 257—262.

A series of adrenergic α- and β-blockers have been found to decrease the release rate of noradrenaline from a suspension of bovine splenic nerve granules incubated in phosphate buffer at pH 7.5. The same blockers also inhibited the uptake of noradrenaline in partially depleted nerve granules in the presence of ATP—Mg. It is tentatively suggested that the interactions of the adrenergic blocking agents with uptake mechanisms for noradrenaline in the nerve granules and at the receptor level in effector cells involve certain common features.

In the course of a study of various autonomic drugs on the release rate of noradrenaline (NA)[1] from isolated bovine splenic nerve granules incubated in isotonic potassium phosphate at pH 7.5, it was observed that phenoxybenzamine (PBA) caused a marked inhibition of the spontaneous NA release and also inhibited the ATP-dependent uptake of NA in partially depleted nerve granules. Even stronger effects were noticed with dichloroisoproterenol (DCI) and chlorpromazine (Euler and Lishajko 1961, 1965). In the present paper we wish to report the action of a number of adrenergic blocking agents, both of the α and β receptor blocking series on the release rate of NA and on the ATP dependent amine uptake.

Methods

Nerve granules were obtained from bovine splenic nerves either by squeezing the desheathed nerves between nylon rollers or by homogenization with an Ultra Turrax apparatus (Janke & Kunkel, Staufen i. Br.). After removal of coarse tissue material and larger particles by centrifugation 10 min at 9 000 × g the granule suspension in the supernatant was incubated at 20° C in 0.13 M potassium phosphate buffer at pH 7.5 for 30—90 min with and without the blocking drugs in concentrations as stated in the experimental part.

[1] Abbreviations used: NA, noradrenaline; A, adrenaline; PBA (phenoxybenzamine); DCI (dichloroisoproterenol).

TABLE 1 Relative release rate (control = 1) for NA in bovine splenic nerve granule suspension incubated for 60 min at 20 C in phosphate buffer, pH 7 5 Mean of 2—6 expts

	Molar concentration 2 × 10 [illegible]	3 × 10 [illegible]
α-receptor blocker		
SY 28		0 30 (0 25—0 35)
Phenoxybenzamine		0 32 (0 24—0 43)
Azapetine (Ilidar)		0 33 (0.29—0 39)
Phentolamine		0 40 (0 14—0 84)
Hydergin	0 30 (0 20—0 35)	
Chlorpromazine	0 47 (1 expt.)	
β-receptor blocker		
Propranolol		0 38 (0 15—0 58)
H 56/28		0 42 (0 33—0 61)
DCI		0 42 (0 30—0 56)
Pronethalol		0 49 (0 42—0 54)
Butoxamine		0 54 (0 36—0 79)
N isopropyl methoxamine		0 56 (0 35—0 79)
MJ 1998		0.86 (0 77—0 94)
MJ 1999		1 2 (1 1 —1 2)

In the uptake experiments the nerve granules were first partially depleted by incubation for 10 min at 37 in phosphate buffer at pH 7 5 and then further incubated for 30 min at 20° in the presence of NA (6 × 10 [illegible] M) ATP (3 mM) and Mg (3 mM) with and without the drugs

Since the NA release follows an approximately exponential course the relative release rate of NA in the presence of the drugs may be expressed as the ratio

$$\frac{\log NA_0 - \log NA_D}{\log NA_0 - \log NA_C}$$

where NA_0 is the original amount of NA NA_D is the remaining NA in the presence of the drug and NA_C the remaining NA in the control after incubation The action of the drugs on the uptake of NA is expressed as the ratio of the NA uptakes with and without the drug Uptake is taken as the net difference in NA values between the sediments before and after the second incubation for 30 min at 20

The following drugs were used

Phenoxybenzamine (Dibenyline® Smith Kline & French Philadelphia) phentolamine (Regitine® CIBA, Basel Hydergin ® (Sandoz Basel SY — 28 (N α naphthylmethyl N-ethyl β bromoethylamine (Parke, Davis & Co. Ann Arbor azapetine phosphate (Ilidar Hoffmann la [illegible]

[illegible]

gifts of the drugs.

Results

Effects on the NA release rate

The α blockers tested, PBA phentolamine, Hydergin, azapetine, SY 28 and chlorpromazine, all lowered the NA release rate from isolated splenic nerve granules as seen in Table 1 On a molar concentration basis Hydergin had the strongest effect while chlorpromazine was least active in this respect

TABLE II Relative uptake of NA in partially depleted bovine splenic nerve in the presence of 3 mM ATP and 3 mM $MgCl_2$ and various adrenergic blockers Mean and range (control uptake = 1)

	Molar concentration 2×10^{-5}	3×10^{-4}
α receptor blocker		
Azapetine		0 10
Phentolamine		0 38 (0 37–0 38)
SY 28		0 61
Phenoxybenzamine		0 62 (0 57–0 68)
Chlorpromazine	0 46	
Hydergin	0 60 (0 53 0 66)	
β-receptor blocker		
Pronethalol		0 19 (0 07–0 25)
DCI		0 22 (0 08 0 37)
Propranolol		0 25 (0 22–0 28)
H 56/28		0 31 (0 30–0 31)
Butoxamine		0 45
MJ 1999		0 73
N isopropyl methoxamine		0 74 (0 66 0 81)
MJ 1998		0 93

Of the β blockers tested (Table I) all had an inhibitory effect on the release rate except MJ 1999, while MJ 1998 had a weak action only The strongest actions were seen with propranolol, 56/28, DCI and pronethalol at a concentration of 3×10^{-4} M

Effect of adrenergic α and β blockers on the NA uptake in partially depleted granules in the presence of ATP Mg

The results obtained with various adrenergic blocking substances on the release of NA from isolated nerve granules made it of interest to study also their effect on the amine uptake As shown previously (Euler and Lishajko 1963) addition of ATP greatly enhances the amine uptake in partially depleted nerve granules and we have studied the effects of the drugs on this stimulated uptake

As seen in Table II all of the blockers inhibited the uptake of NA in the presence of ATP Mg All drugs except Hydergin and chlorpromazine were tested in a concentration of 3×10^{-4} M which caused a maximal or near maximal inhibition of the spontaneous release Hydergin was not tested in a higher concentration than 2×10^{-5} M since this concentration was highly effective on the inhibition of spontaneous release

As seen in Table II the strongest inhibitory effect on the NA uptake was exerted by the α blocker azapetine and by the β blocker pronethalol The β blockers N-isopropylmethoxamine MJ 1998 and MJ 1999 had only a weak effect [illegible] P and Hydergin

On preincubation of the granule suspension for 30 min at 2° C with the drugs, the inhibitory effect on the uptake of NA was greater. The most marked effects were then observed with DCI, propranolol and pronethalol.

Discussion

A wide variety of drugs with different kinds of action have been found to influence the amount of tritiated NA in various organs after injection (Axelrod, Whitby and Hertting 1961). By administration of the drug before or after the radioactive NA certain conclusions could be drawn as to whether the drug caused a release of NA or inhibited the uptake (Axelrod, Hertting and Potter 1962). From these studies it emerged that among several drugs, chlorpromazine and DCI blocked the entry of NA without causing a release.

From the present, extended study it can be seen that inhibition of NA release from splenic nerve granules as well as inhibition of the ATP-dependent uptake is a common property of almost all of the adrenergic receptor blocking substances studied. The term "ATP-dependent uptake" represents approximately the NA uptake in the presence of ATP-Mg, since the NA uptake without addition of ATP-Mg is small under the prevailing conditions.

No strict parallelism was observed between the effect of the drugs on the release rate and that on the NA uptake. Thus Hydergin had a more marked effect on the release rate than on the uptake, and the same was noted for N-isopropyl methoxamine. The latter compound has been reported to be less active than, for instance, pronethalol (Hardman, Barboriak and Meester 1963) in its β blocking action in vivo. MJ 1998 and 1999 have been the only adrenergic receptor blockers so far studied which exert only a negligible action on the granules. Whether this is to some extent associated with their reportedly low lipid solubility is still unclear (Kvam, Riggilo and Lish 1965). No definite relationship between the pharmacological or clinical effects of the various blockers and their inhibitory action of the NA release rate and uptake in splenic nerve granules has been shown to occur.

The two chemically closely related compounds pronethalol and propranolol both have very marked inhibitory effects on the NA release rate as well as on the NA uptake in the presence of ATP-Mg. With regard to their actions in vivo propranolol is reported to have some 5—10 times stronger action than pronethalol.

If in fact there is more than a fortuitous connection between the adrenergic blocking function and the effect on the NA release rate or on the ATP-dependent amine uptake in isolated granules it is tempting to relate it to a common effect on a transmitter uptake in which possibly ATP is involved. The importance of ATP for the uptake of NA has been demonstrated in different ways. Addition of ATP to the incubation medium diminishes or prevents the spontaneous net loss of NA from nerve granules, owing largely to a compensatory uptake of NA when this amine is present. Furthermore, after partial depletion of NA from the granules following incubation, ATP greatly enhances the uptake of exogenous NA (Euler and Lis-

hajko 1963), as observed also for adrenal medullary granules (Carlsson Hillarp and Waldeck 1963)

The protective effect of NA and ATP by which the NA content of the granules is maintained during incubation, is to some extent counteracted by PBA, which although it strongly lowers the spontaneous release rate to about 1/8 of the control rate in a concentration of 7.4×10^{-4} M exerts a smaller "protective" effect at this concentration than at 3×10^{-4} M This may be explained by assuming that the protective effect is dependent on an uptake which is inhibited by PBA A still stronger inhibition of the "protective" effect of ATP was observed in the present study with propranolol 3×10^{-4} M which removed about 80 per cent of the ATP effect

As to the spontaneous NA release from granules Euler Lishajko and Stjärne (1963) have reported that the amine release is relatively faster than the accompanying ATP release which causes a conspicuous fall in the amine ATP ratio as incubation proceeds Preliminary experiments seem to indicate that the drugs under study alter this ratio (Euler and Stjarne unpublished obs)

An interaction of adrenolytic agents with uptake of NA in organs has been previously observed (Axelrod *et al* 1961, 1962 Rosell and Axelrod 1963) and it has been assumed that such agents prevent the binding and subsequent destruction of the released transmitter at the receptor level (Brown and Gillespie 1957 Schapiro 1958) The increased overflow of transmitter from the spleen has also been interpreted as inhibition of reuptake (Blakeley Brown and Geffen 1964 Kirpekar and Cervoni (1963) have concluded from their experiments with various adrenergic blocking agents that the tissue receptors are involved in a major way in inactivating neurally released transmitter On the other hand Burgen and Iversen 1965 find no support for the view that the uptake of catecholamines is explicable in terms of a binding at sympathetic α or β receptors

The present observations appear to give the first indication of a common antagonistic effect of a specific group of pharmacologically active substances on the isolated granule system and on effector cells The results are thus suggestive of an NA uptake mechanism bound to receptors both in granules and in effector cells which can be blocked by the same kind of substances Whether or not the uptake of NA in the receptor of the effector cell is ATP dependent is not experimentally proven but it has been postulated Belleau 1960 that ATP might have an important function in this system.

Should a functional similarity in the NA uptake mechanism between nerve granules and the adjacent synaptic receptor [illegible] this might conceivably reflect some association between the effector cell and [illegible] out growing adrenergic axons which make contacts at an early stage during development

The research reported in this document [illegible] sponsored in part by the Air Force Office of Scientific Research under Grant AF EOAR [illegible] through the European Office of Aerospace Research OAR United States Air Force and by [illegible] Swedish Medical Research Council [illegible]
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Types of Muscle Fibres in Toad Skeletal Muscle

By

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Received 12 April 1966

Abstract

LANNERGREN, J. and R. S. SMITH. *Types of muscle fibres in toad skeletal muscle.* Acta physiol. scand. 1966. *68.* 263—274.

An attempt has been made to define the types of muscle fibres present in the iliofibularis muscle of *Xenopus laevis*. Histochemical studies were carried out on cross sections of the muscle. The variation between cells in the content of lipids, DPN diaphorase and succinic dehydrogenase was noted, and on this basis the fibres were classified into three main groups. Single living fibres representing the histochemical groups were selected and their contractile behaviour in response to electrical stimulation and the application of ACh was studied. The findings indicated a relationship between the histochemical and functional properties of the muscle fibres. Fibres with a moderate content of lipid and enzymes (pale fibres) were fast fibres which fatigued rapidly on [illegible]
Fibres with a high content of lipid and enzymes (dark [illegible]
rapidly. Fibres containing no lip[illegible]
were slow fibres. Staining of the [illegible]
of varying gross morphology an[illegible] ... en
grappe end plates with very low [illegible]

Amphibian skeletal muscle is generally regarded as being composed of two types of muscle fibres, fast and slow (Tasaki and Mizutani 1944, Kuffler and Gerard 1947). A stimulus which depolarizes a fast fibre to or beyond a threshold causes a propagated electrical impulse and a twitch. Slow fibres, however, show no regenerative electrical activity and respond to a local depolarization with a local contraction (see review by Peachey 1961). Structural differences between fast and slow muscle fibres have also been described (Page 196[illegible]). The evidence which indicates the presence of these two groups of muscle fibres does not preclude the existence of sub-types within either group of fibres, nor even of the existence of entirely unrecognized groups of muscle fibres. Shamarina's (1962, 196[illegible]) description of fibres with properties intermediate between those of fast and slow fibres can be taken to be a case in point, although the validity of these results has been doubted (Orkand 1963).

The object of the present investigation was to determine the function[illegible] muscle fibres present in a toad skeletal muscle. First, the histochemi[illegible]

of all the fibres in the muscle were determined from cross sections of the muscle, and the fibres were separated into histochemical groups Fibres representing the histochemical groups were isolated and their contractile behaviour was investigated It will be shown that in *Xenopus laevis* the contractile properties of individual fibres can be related to the histochemical properties of the fibres

Fast and slow fibres have distinctly different histochemical properties, and the fast group of fibres may be subdivided on both histochemical and functional grounds This is consistent with an earlier finding in the frog *R ridibunda* (Lannergren 1965)

Methods

The experiments were performed if not stated otherwise on the iliofibularis muscle of the toad *Xenopus laevis* Both male and female toads were used of weights ranging between 40 and 80 g The animals were kept at room temperature and were fed minced meat once a week Histological investigations were performed on muscles excised between January and September while the observations of the contractile behaviour of the muscle fibres were made during July—September

Histological procedures

Cross sections of muscle were stained for lipids succinic dehydrogenase and diphosphopyridine nucleotide-diaphorase (DPN-diaphorase The procedures used were

(a) Lipids were stained with Sudan Black B (Carleton and Drury 1957) Staining was carried out partly on 5 μm sections of tissue which had been fixed in formalin and embedded in Carbowax and partly on 16—20 μm sections of frozen fresh tissue

(b Succinic dehydrogenase was demonstrated by the method of Nachlas *et al* (1957

(c DPN-diaphorase was demonstrated using the method of Scarpelli Hess and Pearse as described by Pearse 1960 Nitro-BT was used as the electron acceptor

Prior to the procedures (b and c the animals were cooled to about 0 C then pithed and an iliofibularis muscle was excised The muscle was then immediately frozen in liquid propane at — 190 C and subsequently stored at — 65 C for periods not longer than two weeks Serial 1[illegible] 20 μm sections were cut on a cryostat and alternate sections were used for demonstrating enzymes or lipids.

Cholinesterase at the end plates of single muscle fibres or on small bundles of fibres was demonstrated by the thiolacetic acid method at pH [illegible] Barnett and Palade 1958

Nerve terminals were stained using a Rongalite methylene blue method Romeis 1928 on isolated muscle fibres

Single fibre dissection

The excised iliofibularis muscle was held by its tendons between split Nylon rods in a shallow Perspex dish containing Ringer's solution Fig 1 Single muscle fibres were then isolated with the aid of finely ground forceps and scissors

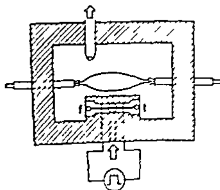


Fig 1 Schematic drawing of the cell used for dissection and recording The muscle was held between split Nylon rods during the dissection of a single fibre After dissection the fibre was attached to the glass rods *f* and *t* and moved in the Ringer's solution to a gutter where a cover-slip was placed over it The rod *f* was fixed directly to a micromanipulator the rod *t* was attached to the movable plate of a transducer which was mounted on a second micromanipulator Flow of Ringer's solution indicated by arrows

A condensor system as described by Huxley, Kearney and Purvis (1962) was used to give dark field [illegible]
of [illegible]
be [illegible]
sco[illegible]
microscope [illegible]

[illegible]

[illegible] muscle [illegible]

One of the glass rods [illegible] (Fig 1) was fixed to a micromanipulator while the second rod [illegible] was fastened to the movable plate of a variable-capacitance mechano-electric transducer The trans- [illegible]

[illegible] fibre was [illegible] uge had [illegible] ity and [illegible] nt also
the diameter of the muscle fibre was measured to the nearest 5 µm di[illegible]ision on an eye-piece micrometer Several readings were taken along the length of the fibre

Ringer's solution

The Ringer's solution had the composition (mM) NaCl 112 0 KCl 2 5 $CaCl_2$ 2 0 $NaHCO_3$ 2 [illegible] Na_2HPO_4 1 2 NaH_2PO_4 0 6 and glucose 5 5 When saturated with 99 % O_2 and 1 % CO_2 the pH of the solution was 7 2—7 4 The solution bathing the fibre was changed periodically by allowing fresh solution to enter at the centre of the gutter which contained the fibre (see Fig 1 while the excess flu d was removed by suction The experiments were carried out at room temperature 21—25 C)

Results

Cross sections of the iliofibularis muscle showed a characteristic pattern of staining that was similar whether the sections were stained for lipids or for the enzymes DPN diaphorase or succinic dehydrogenase Plate 1A demonstrates the pattern in a section stained with Sudan Black B Regardless of the staining method used the muscle showed a peripheral zone composed mainly of large diameter fibres containing a moderate number of stained granules pale fibres and an eccentrically placed inner zone of fibres of smaller diameter which were densely packed with stained granules dark fibres) In the centre of the mass of dark fibres a number of fibres were found in which few or no granules could be demonstrated with any of the three techniques (clear fibres By staining serial sections with the three techniques the staining properties of single cells could be examined Plate 2 The results of such examination are given in Table I

Sections of the [illegible] an Black B This muscle [illegible] were [illegible] his animal [illegible] were examined [illegible] extensor longus digitorum IV contained large pale fibres small dark [illegible] The [illegible] muscle obtained large pale fibres smaller dark fibres [illegible]

In summary it was found that [illegible] of th[illegible] lipid and enzyme contents the skeletal muscle fibres of the toad [illegible] ld be separated into three fairly distinct gro[illegible] pale dark and clear fibres A number [illegible] fibres were found in the centre [illegible]

A

0 5 mm

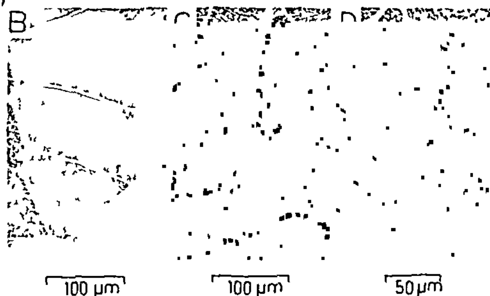


PLATE 1

A Cross-sect n of a whole il f bularis n uscle fixed in formal n en bedded in Carbowax a 1

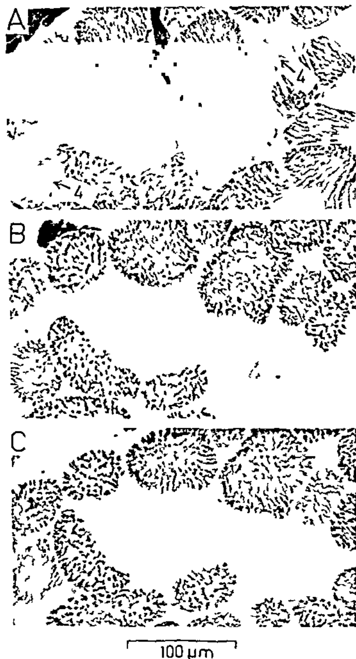


PLATE 2

Frozen 20 μm serial sections from centre of dark zone of I. fibularis muscle. *A*. Section stained with Sudan Black B. *B*. DPN-diaphorase staining. *C*. Succinic dehydrogenase staining. Alternate sections are stained allowing the same fibre to be followed through the sections. Four nonstained fibres can be seen in the centre of all sections and can be used for orientation. Two fibres of the type in column 4 of Table I are indicated by arrows and number 4.

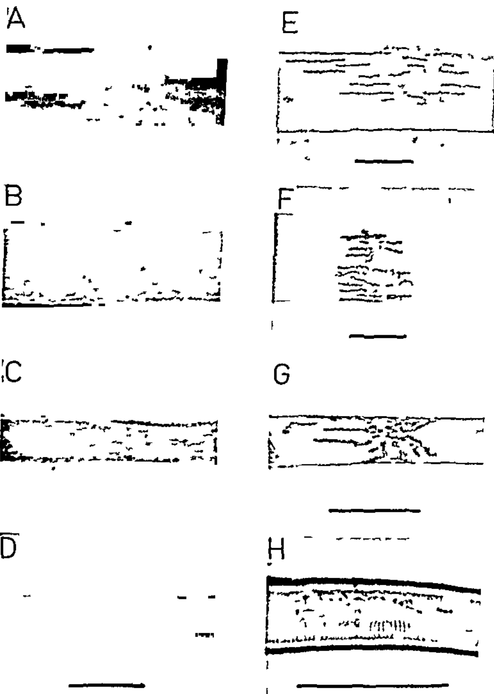


PLATE 3

A, B, C and *D*. Appearance of single living muscle fibres in dark field illumination. [illegible] of the types described in Table 1, column 1, 2, 3 and 5 respectively. *E, F* and *G*. End-plates [illegible] corresponding fibres stained by the thiolacetic acid method. *H*. Nerve ending [illegible] graph [illegible] on a clear fibre (Table 1, column 5). Methylene blue. Oblique illumination. Fibres *E, F* [illegible] *H* flattened between slide and coverslip. Scale is the same for *A* to *D*. Scale bars 10[illegible]

TABLE I Properties of the different muscle fibres in the iliofibularis muscle s laevis

	1	2	3	4	5
Location (p = peripheral c = central)	p	c	c	c	
Approx no of fibres	350	200	50	5[illegible]	
Diameter in μm	100–150	55–110	40–70	3	
Affinity to stain					
Sudan Black B	+	+++	++	(	
DPN-diaphorase specific method	+	+++	++	–	r
SDH-specific method	+	+++	++	– –	r
Nomenclature	pale	dark	dark	—	

zone of the iliofibularis muscle which, to some extent, deviated from scheme The properties of the deviant fibres are given in columns 3 and 4 o In Plate 2 two fibres of the type in column 4 are indicated by arrows and numbe

Selection of single living fibres representing the histochemical groups

Individual living fibres isolated from the iliofibularis muscle were found to scatter light to different extent when observed in dark field illumination Fibres from the peripheral zone of the muscle (pale fibres) were found to scatter very little light (Plate 3A), while the majority of the fibres in the dark zone scattered much light (Plate 3B) In the centre of the zone of dark fibres a number of fibres were found which scattered no light (Plate 3D)

The light-scattering properties of single muscle fibres presumably depend on one or more of the structures in the sarcoplasm In view of the histochemical findings it seems possible that fat droplets and mitochondria either separately or together may contribute to scattered light It is also possible that the myofibrillar arrangement may be a contributing factor The myofibrillar arrangement however may be discounted since scattered light can be seen to originate in punctate sources The conclusion is also supported by some observations of the fine structure of these fibres which show that the myofibrillar arrangement of pale and dark fibres is similar (fibrillar structure) Thus the most likely source of scattered light would seem to be fat droplets and mitochondria, and of these fat droplets are the most probable source owing to their refractivity If fat droplets alone give rise to scattered light then the sections stained with Sudan Black B are likely to represent a 'map' of the light scattering properties of the fibres in the muscle If both fat and mitochondria contribute towards light scattering a composite effect will be obtained, the magnitude of which can be estimated from Table I since both DPN-diaphorase and succinic dehydrogenase specific granules in the muscle may be taken to represent mitochondria In any case, the selection of fib

columns 1, 2, 3 and 5 should have been reasonably unambigous Fibres of the type shown in the table, column 4, however, may have been included with the fibres of column 3 or with those of column 5

In these experiments four groups of fibres were selected as shown in Plate 3 ABCD, representing the fibres of Table I, columns 1, 2, (3 and 4) together, and 5 or, the fibres of columns 1, 2 3, and (4 and 5) together

End plate morphology

It has been suggested that muscle fibres with different types of end plates also differ in other properties (e g Cole 1957, Hess 1960 1961 Hess and Pilar 1963) The presence of end plates of the en grappe type is a part of the morphological description of amphibian slow muscle fibres (Gray 1956 Hess 1960 Page 1965) while the fast fibres have end plates showing the well known arborizing pattern (e g Hess 1960)

End plates were examined on the four groups of fibres as selected by their light scattering properties with the object of detecting any systematic variation in end plate morphology

Single fibres or small bundles of fibres were dissected out their light scattering properties were determined and end plates were stained using the thiolacetic acid method for cholinesterases Plate 3 E F and G shows such stained end plates The pictures of Plate 3 are mounted so that the two muscle fibres of each row were from the same region of the muscle and have the same light scattering properties It is apparent that individual end plates on the pale and dark fibres do show differences in structure those in the dark region of the muscle being shorter with a tendency in some fibres to be bushy as in Plate 3 G The pale and dark fibres had one two or three end plates two being most common

The small diameter fibres which scattered no light Plate 3 D) and which were situated in the centre of the zone of dark fibres failed to show any end plates with the thiolacetic acid method even though the incubation times were extended until the muscle tendon junctions stained deeply This result leads to the conclusion that the cholinesterase activity at the end plates of these fibres was very much lower than in any of the other fibres Nerve terminals on these fibres were successfully stained with the Rongalite methylene blue method Ten such fibres each 6—8 mm in length were stained and all of them showed terminals of the en grappe type Plate 3H There were usually three widely-spaced endings in each fibre

The contractile properties of single muscle fibres

The contractions of 33 isolated muscle fibres in response to single and repetitive electrical stimulation were recorded Acetylcholine was used as a stimulating agent on a number of these fibres The fibres were classified on the basis of their light scattering properties and their localization in the muscle into four classes Seven fibres were classified as the type in column 1 Table I, seven as the type in column 2

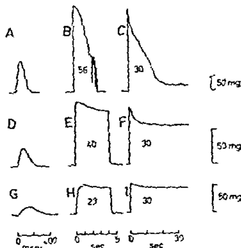


Fig 2 The ulate (A D midd obtain (C, F, lation µm fil e type in column 1 of Table I D E F from a 60 µm fibre of the type in column 2 of Table I and G H I from a 50 µm fibre of the type in column 3 of Table I In *B* the final brief decreases in tension are caused by failure of action potential propagation

thirteen as column 3 type and six as the type in column 5 The latter two classes as explained on p 00, may have included fibres of the type shown in column 4 of Table I

Twitch fibres

The fibre was attached to the strain gauge, as described, and its length adjusted so that the peak tension resulting from a single electrical stimulus was maximal Fig 2 shows tracings of the mechanical responses of these fibres The top row is from an experiment with a large fibre from the peripheral zone of the muscle, i e a pale fibre (Table I, column 1) The middle row of tracings is from an experiment with a dark fibre of the type in column 2 of Table I The bottom row of records was obtained from a fibre of the type in column 3 of Table I

The left hand column in Fig 2 shows the response of the fibres to a single stimulus and indicates that all these fibres responded with a distinct twitch The centre column shows the tension resulting from repetitive stimulation at fusion frequency, while the right hand column shows the tension developed during long lasting stimulation at a frequency of 30 imp sec Stimulation of the fibre at a frequency of 30 imp/sec was chosen as a standard testing procedure for all types of fibres because mechanical fatigue could develop rapidly while the electrical impulses in the cell membrane did not drop out as they may do at higher stimulation rates

Fig 2 and Table II show that there are differences between the three groups of fibres in the time course of the single twitch in the fusion frequency and in the duration of maintained tension during repetitive stimulation The entire collection of fast fibres showed a wide range in the duration of twitch tension Extreme values of 20 and 80 msec for the contraction time were obtained

A very striking difference was found between the peripheral pale fibres central dark fibres in their susceptibility to fatigue The peripheral fibres

TABLE II Characteristics of the mechanical activity of fast fibres of the types described in table I

Column in Table I	1	2	3
Contraction time of twitch (msec)	29 3±2 3 (7)	37 2±2 0 (7)	53 8±4 5 (13)
Peak to half-decay time of twitch (msec)	34 3±1 3 (7)	34 3±2 5 (7)	56 2±3 5 (13)
Max tetanic tension (kg/cm²)	3 3±0 4 (5)	3 1±0 2 (7)	3 1±0 3 (11)
Tetanus to twitch tension ratio	2 0±0 2 (6)	3 0±0 3 (6)	3 7±0 3 (13)
Fusion frequency (imp/sec)	33–60 (5)	33–48 (6)	23–33 (12)
Tension after 30 sec stim at 30/sec (per cent of initial tension)	3–10 (6)	5[illegible]–77 (3)	63–93 (11)

The values given are means ± S E of mean except for fusion frequency and tension after 30 sec stimulation where the range is given
Contraction time is given as the time from the beginning of the mechanical response to its peak
Number in parenthesis indicates number of fibres observed

quickly when stimulated repetitively (Fig 2 C) while the dark fibres resisted fatigue (Fig 2 F, I) The rapid decline of tension occurring in the pale fibres was not due to a failure of propagation of the action potential This was demonstrated by recording tension and external actions currents simultaneously in some of these fibres

The mean maximum tetanic tension of the whole collection of fast fibres was 3 1 ± 0 2 kg cm² mean ± S E of mean , there being no significant difference between pale and dark fibres in this respect

Slow fibres

The small clear fibres Table I, column 5 which were isolated from the centre of the zone of dark fibres responded only with local contractions at the cathode when stimulated electrically If, with the electrode arrangement shown in Fig 1 and the fibre at about resting length a train of stimuli were applied then the resulting tension did not exceed 3 mg or 0 15 kg cm² However, when a Ringer s solution containing 10⁻⁶ g ml acetylcholine was applied then the whole fibre contracted rapidly and held a maintained tension of about 4 kg cm² The fibres relaxed promptly when the acetylcholine was washed away with Ringer s solution the contractures being repeatable Acetylcholine caused the other types of muscle fibres Table I columns 1, 2 and 3) to give a single twitch only

Discussion

The evidence presented here supports the broad division of muscle fibres in anuran skeletal muscle into 'fast and slow groups It is apparent however that the fast group of fibres may be subdivided both on histochemical and functional grounds

At the present time it would seem to be most useful, in describing the fibre types comprising the iliofibularis muscle of *Xenopus*, to say that there are clearly two broad groups of fast fibres and a group of slow fibres One group consists of large diameter fibres which, on the histochemical evidence, contain a moderate amount of fat droplets and mitochondria (pale fibres), display a twitch tension of short duration and do not maintain tension on prolonged stimulation (Table II, column 1)

A second group encloses fast fibres containing many fat droplets and mitochondria (dark fibres), with twitch tensions of longer duration and with maintained tension on prolonged stimulation (Table II, columns 2 and 3) That the group of dark fibres might itself be meaningfully subdivided is possible but not demonstrated by the present experiments The fibres of Table II, columns 2 and 3 might well have been selected from the extremes of a population of fibres with a continuous range of properties The contractile properties of clear fibres were not studied in sufficient detail to enable one to determine whether or not this group of fibres was completely homogeneous in its properties, but it is certain that none of these fibres propagated an impulse As pointed out in the Results, it remains a matter of speculation whether the fibres with the properties shown in Table I, column 4 fall into the fast or slow groups

No fibre that could be said to have properties intermediate between 'fast' and 'slow' was detected, even though more fibres were selected from the vicinity of the slow fibres than from any other part of the muscle (Table I, column 3)

The morphology of the end-plates on the fibre types described above indirectly supports the general conclusions All fibres which were 'fast' had end plates whose forms may be regarded as falling within the overall morphological variation of 'twitch fibre' end-plates On the other hand, there does exist a systematic variation in morphology between the end plates on pale and dark fast fibres respectively

Acetylcholine was applied in some experiments to the various types of fibre Only the very clear fibres in the centre of the muscle responded with lasting contractures The situation in the frog appears to be different (Kiessling 1964)

All clear fibres had end-plates of the 'en grappe' type with undetectable cholinesterase activity with the method used The Rongalite methylene blue method gave very reproducible results and showed, on the average, tree widely spaced clusters of endings on each fibre An estimate of the depolarization of a fibre of 8 mm length, and a characteristic length of 1 mm, by equidistantly spaced end plates shows that a *point mid way between two end plates will be depolarized by an amount which is* about two thirds of the depolarization at an end plate Thus the observation of only three clusters of 'en grappe' endings on each clear fibre is not inconsistent with the idea that these fibres function effectively in the absence of a propagated impulse in the cell membrane

This investigation was supported by grants from Karolinska Institutet ("Reservationsanslaget", "Anslaget till biträdeshjälp m m åt docenter the Swedish Medical Research Council (Project No Y 615) and "Stiftelsen Gustaf och Tyra Svenssons minne

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Acta physiol. scand. 1966. 68. 275—285

From the Institute of Medical Physiology B University of Copenhagen Denmark

The Filtration Fractions of Plasma Supplying the Superficial and Deep Venous Drainage Area of the Cat Kidney

By

OLE I NISSEN

Received 15 April 1966

Abstract

NISSEN, O I *The filtration fractions of plasma supplying the superficial and deep venous drainage area of the cat kidney* Acta physiol scand 1966 *68* 275—285

In 23 expts the plasma concentrations of protein and inulin were measured in blood collected

In a previous paper (Nissen 1965) the occurrence of different plasma protein concentrations in blood samples obtained from the subcapsular veins (by puncture) and the deep veins (by catheterization) of the cat kidney has been reported The concentrations were respectively 9 per cent higher and 11 per cent lower than the concentration of arterial blood taken simultaneously

The discrepancies were interpreted as the result of reabsorption to the deep drainage blood of some of the fluid ultrafiltered from the blood traversing the superficial part of the cortex

In this paper a method is presented which allows a calculation of the filtration fractions of the plasma flows supplying the two venous drainage areas

Anatomy

Many mammalian kidneys (including those of man dog and cat possess two systems of veins a deep and a superficial (Fig 1) The former which drains the inner part of the cortex and the medulla has a course similar to that of the arterial system However two essential differences exist 1) the veins corresponding to the interlobular arteries the inner cortical veins) begin "blindly"

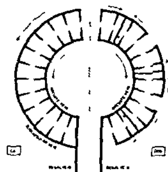


Fig 1 *Left* The two vein systems of the *cat* kidney Blood from the superficial cortex to the *renal vein* passes the *outer cortical* and the *subcapsular veins* Blood passing from the deep cortex (and the medulla — vessels not figured) to the renal vein passes the

the cortex

the arterial system

Theory

The arterial inflow and the venous outflow of plasma of a drainage area in the kidney is called ARPF and VRPF

The VRPF of an area may differ from that of its ARPF because the rate at which plasma fluid is lost by ultrafiltration in the area may differ from that by which fluid is regained by reabsorption

The filtration fraction (FF) is defined as the filtration rate (F) divided by arterial plasma flow FF = F/ARPF A_t is the plasma concentration of inulin in arterial blood, V_t the plasma concentration of inulin in the renal venous blood from the area in question f_w (= 1 05) is a factor correcting a plasma concentration to the concentration in plasma water

For the area in question the inflow of inulin in the arterial plasma minus the amount of inulin filtered is equal to the amount drained away by the venous plasma flow

$$ARPF \; A - (FF \; ARPF) \; (A \; f_w) = VRPF \; V_t$$

$$\text{or } 1 - FF \; f_w = \frac{VRPF}{ARPF} \cdot \frac{V_t}{A_t} \qquad (1$$

* This was tested experimentally in the cat A polyethylene catheter the same type as used in the remaining experiments) was inserted through one of the two branches of the renal vein into the deep veins The pressure was 9—14 mm Hg (as compared to 4 5 mm Hg in the caval vein When a ligature was tightened around the branch with the catheter the pressure only rose 5 mm Hg

As protein (P) for all practical purposes is not filtered in the glomeruli we obtain

$$\text{ARPF} \cdot A_P = \text{VRPF} \cdot V_P$$

$$\text{or } \frac{\text{VRPF}}{\text{ARPF}} = \frac{A_P}{V_P} \quad (2)$$

Inserting 2 in 1 yields

$$\text{FF} = \frac{1}{f_W}\left(1 - \frac{V_I}{A_I} \cdot \frac{A_P}{V_P}\right) = \frac{1}{f_W} \cdot \frac{\frac{V_P}{A_P} - \frac{V_I}{A_I}}{\frac{V_P}{A_P}} \quad (3)$$

V_X/A_X is called the *fractional concentration* of X

Discussion In setting up formula 1 it is presumed that *inulin* is completely filtrable in the glomerulus and that it is neither synthesized, destroyed excreted or reabsorbed by the tubule These "classical requirements to inulin are discussed by Smith (1951) In some of my experiments both inulin and creatinine were used as "indicator substance to compare the results of creatinine with those of inulin

I have investigated this claim using an improved technique The results constitute a part of the work presented in this report

The formulas 1 and 2 are only correct if a steady state exists for the renal interstitial fluid volumes

care was taken to maintain constant arterial pressure and constant arterial concentrations of inulin (and creatinine) during the experiments

The renal *lymph flow* is small approximately half the normal urine flow (Mayerson 1963) which is again roughly 0 2 per cent of the plasma flow Furthermore this fluid is not inulin and protein free according to Kaplan Friedman and Kruger (1943) and Mayerson (1963) the protein and inulin concentrations are approximately 0 5 and 0 7 times the arterial concentrations Both facts indicate that the lymph flow constitutes only a minor source of error in the experiments No attempts were made to correct this error

Method

The experiments were performed on 26 cats averaging 4 5 kg in weight (range 3 0—6 8) They were anesthetized with pentobarbital 40 mg/kg i p plus supplementary doses i v or with chloralose 60—90 mg/kg i p The animals who in the days prior to the experiment were fed libarately with meat and fish were fasted for 14—18 hours prior to the experiments These were carried out with the animals placed in the supine position on a heated table The arterial blood pressure was measured in a carotid or femoral artery Approximately 25 mg heparin was injected just before taking the blood samples

Collection of arterial and mixed renal venous blood Degradation of protein in the kidney 3 expts in chloralose anesthesia) The arterial blood was sampled from a carotid artery In order to ensure that the blood from the two venous drainage areas was well mixed before collection the blood was first led through 6—8 cm of a PVC tube through which 4 6 pins had been thrust to produce turbulence The tube (i d. 3—4 mm) connected the inferior caval vein just distal to the kidneys with the same vein proximal to these This by pass produced minimal stasis of the kidneys While inserting the PVC tube the renal blood was temporarily led through a side branch of the tube to a jugular vein to avoid any interruption of the renal blood flow The blood from the inferior extremities was led to the other jugular vein

The two samples were collected simultaneously by a multi tube roller pump (Desaga) at rates of 0 15—0 30 ml per

2 × 3 5 ml were collected During each sampling period the diuresis and the renal plasma flow were determined, thus the rise of the fractional protein concentration of mixed renal venous plasma caused by loss of urine could be allowed for RPF was measured as the excretion rate PAH divided by A_{PAH}—V_{PAH}, where V_{PAH} in this case is the PAH concentration in the mixed renal venous plasma

ing

To facilitate the insertion a 0 5—1 0 mm long incision was made in the tough fibrous capsule

ureter, in 4 expts the ureter was catheterized to keep the urine clear of the slight hemorrhage arising from the divided ureter

In all the experiments a priming dose followed by a constant infusion (0 4—0 5 ml/min) of inulin in 0 9 per cent saline was given in a jugular vein In addition in some experiments creatinine glucose phloridzin p aminohippurate sodium or potassium-phosphate was administered The results from a few periods in which an osmotic diuresis exceeding 0 2 ml/min from the experimental kidney was provoked are excluded the subject of osmotic diuresis is treated in a subsequent paper Small doses of donor blood were given if the blood pressure showed a tendency to fall

At the end of the experiment the kidney was inspected to determine the position of the deep catheter and to disclose any lesion of the kidney tissue made by the catheter A lesion in the superficial cortex was observed only once (the corresponding data were discarded) in the deep regions of the kidney no lesions were observed

Analyses The *protein* was determined in duplicate by a biuret reaction (Gornall Bardawill and David 1949 using 100 µl plasma for each determination The standard deviation (SD) of the single determination determined from duplicates (SD $\sqrt{\frac{\Sigma d^2}{2n}}$) was 0 6 per cent of the mean (2n 10

All deproteinizations were made in duplicate two determinations were made from each deproteinized sample

Inulin was analyzed by the method of Bojesen 1952) using 1 5 the volume of the original procedure 100 µl plasma was used for each deproteinization The coefficient of variation of the single deproteinized sample was 0 8 per cent (2n 156) Glucose brought about an extinction of 1 per cent of that of inulin a correction for this was applied with low inulin concentrations or high glucose concentrations or both

Creatinine was analyzed according to Peters (1942) modification of Jaffe s reaction using 500 µl plasma for each deproteinization The coefficient of variation was 1 0 per cent (2n 152 The plasma recovery was determined for each analytical batch normally it was between 87 and 90 per cent (range 85 100

Glucose was analyzed by an enzymatic method (Bergmeyer 1963 using 100 µl plasma per deproteinization The coefficient of variation was 0 6 per cent 2n 154) Fluoride admixture to the blood samples was not used as the salt would produce osmotic reduction of the red cell volume thus producing a plasma dilution To minimize the ensuing degradation of glucose the samples were centrifuged immediately after the period

The coefficient of variation due to the analytical error) on the final expression FF$_D$ FF$_S$ (cf appendix) was of a reasonably low value (about 4 per cent firstly because the determinations were always performed in duplicates this reduces the coefficient of variation of the protein and inulin determinations by the factor 1 √2 in the double determinations Secondly because the venous and arterial plasma concentrations used in the FF expression were always derived from the same analytical batch (and thus allowing the standard deviation to be calculated from the duplicates

Calculation

The fractional SD of the expression

$$FF\ f_N\quad \frac{V_P\ A_P\quad V_I\ A_I}{V_P\ A_P} = 1\quad \frac{V_I\ A_I}{V_P\ A_P}$$

may be calculated using 0 30 as a representative value for this quantity and 0 08 as a suitable coefficient of variation of both analyses

The fractional SD of V_I/A_I and V_P/A_P is

$$\sqrt{\left(\frac{0.008}{\sqrt{2}}\right)^2 + \left(\frac{0.008}{\sqrt{2}}\right)^2} = 0.008$$

The fractional SD of $(V_I/A_I) \cdot (V_P/A_P)$ is

$$\sqrt{(0.008)^2 + (0.008)^2} = 0.008 \cdot \sqrt{2}$$

The "absolute" SD of $1 - (V_I/A_I) \cdot (V_P/A_P)$ is

$$0.70 \cdot (0.008\sqrt{2})$$

and the fractional SD of the same expression

$$\frac{0.70 \cdot 0.008 \cdot \sqrt{2}}{0.30} = 0.026$$

In the final ratio FF_D/FF_S the factor $1/f_W$ cancels out. So the fractional SD of FF_D/FF_S will be

$$\sqrt{(0.026)^2 + (0.026)^2} = 0.026 \cdot \sqrt{2} = 0.037$$

Results

Collection of arterial and mixed renal venous blood. Degradation of protein in the kidney. The fractional protein concentration (V_P/A_P) was determined in 32 periods of 3 experiments. In 23 the blood pressure varied at the most 20 mm Hg within the period, and the blood pressure level was evidently above the lower limit of the autoregulatory range of the renal blood flow. When these conditions were not fulfilled the number is given in brackets.

First experiment: 1.005, 1.007, 0.993, 1.001, 1.005, 0.995, 0.989, (0.986), (0.996), (0.972).

Second experiment: 0.994, 0.989, 0.976, 0.994, 0.987, 0.982, (0.993), (0.993), (1.002), 1.015), (1.023), (0.990).

Third experiment: 1.003, 0.998, 1.002, 0.997, 1.001, 1.000, 0.990, 0.987, 1.003, 0.997.

The average figure for the 23 periods was 0.995. The correction for the loss of urine, to be subtracted from the fractional concentrations, averaged 0.002 in the 3 experiments.

Collection of arterial blood and of venous blood from the two drainage areas. 1. Comparison between the handling of inulin and creatinine in the kidney. The results are derived from 39 periods in 10 experiments. The average clearance of inulin for the experimental kidney was 7.6 ml/min (range 2.3—13.8). The average creatinine to inulin clearance ratio was 0.94, SD 0.10 (n = 37). It is significantly lower than 1.00 (p < 0.001, Student's t).

A reasonable agreement existed between the creatinine to inulin clearance ratios and the ratios between the creatinine extraction fractions and the inulin extraction fractions for the two drainage areas [illegible]. Thus the mean ratio for the superficial area was 0.94, SD 0.03 (n = 39), and for the deep area 0.90, SD 0.07 (n = 37). A value of 2.3 was omitted in the calculation of the former mean. The means are significantly lower than 1.00 (p < 0.001).

The range of the arterial plasma concentrations of inulin was 11—83 mg/100 ml (mean 39). The range [illegible] 27—82 mg/100 ml (mean 48).

2. *The filtered fractions.* FF_S and FF_D of the two drainage areas, calculated from 75 periods in 23 experiments, are presented in Fig. 2a. The average FF_S was 0.34. For FF_S values in the range 0.1—0.2 the mean FF_D/FF_S ratio was 0.7. For FF_S-values in the ranges 0.2—0.3, 0.3—0.4 and 0.4—0.5 the corresponding mean FF_D/FF_S ratios were 0.80 ± 0.02 (SE), 0.86 ± 0.03 (SE) and 0.88 ± 0.02 (SE), the 3 last means were significantly lower than 1.00 ($p < 0.001$). The average FF_D/FF_S for all the periods was 0.85.

In 10 experiments (43 periods) both creatinine and inulin was infused. The FF_D/FF_S calculations based on the creatinine (and protein) concentration yielded an average value of 0.74 (Fig. 2 b).

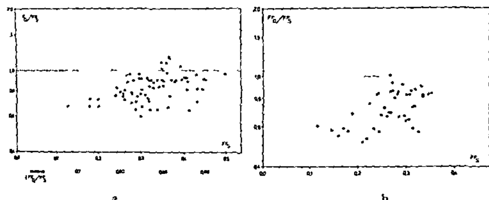
Discussion

Degradation of protein in the kidney. The experiments showed an average fractional (venous/arterial) plasma protein concentration very near to *1.000 (0.995)*. This finding is in agreement with Lathem and Benjamin's (1956) observation that no a.-v. differences for protein could be demonstrated in the dog kidney. Considering the magnitude of the renal plasma flow the results are consonant with the facts that the degradation rate of plasma protein in the whole organism is only about 0.3 g/kg × day (*cf.* for example Jarnum 1963) — and that the degradation occurs only to a minor extent (below 10 per cent) in the kidneys (Katz, Rosenfeld and Sellers 1960, Katz, Sellers and Bonorris 1964). Thus protein is not degraded in the kidney to such an extent that it invalidates the use of the fractional protein concentration in the formulas.

The reason for renal a.-v. differences of 1—5 per cent of the arterial concentration measured by other authors (Gerbi 1951, Harms *et al.* 1962) is probably a dilution of the renal capillary blood by interstitial fluid. The cause of dilution is the withdrawal of rather large blood samples, resulting in a fall in arterial and renal venous pressure (blood is collected from the renal vein). Renal vasoconstriction may also cause a fluid displacement from the renal interstitial spaces to the capillaries. A dilution was obtained in dogs in exaggerated form by Heimburg and Ochwadt (1961) by lowering of the blood pressure in the abdominal aorta or by i.v. injection of adrenalin.

Comparison between the renal handling of inulin and creatinine. One group of authors (*cf.* Smith 1951) has obtained rather good agreement between inulin and creatinine clearances in dogs and cats. Agreement, however, was not obtained by Eggleton and Habib (1951) and Peters (1963). With low inulin concentrations Eggleton and Habib (1951) found that the inulin clearance was the larger, with higher concentrations the creatinine clearance was the larger. Opposite results were obtained by Peters.

Ladd, Liddle and Gagnon (1956) obtained a mean creatinine to inulin clearance ratio, significantly below 1.00 (0.94, SD 0.05) in the dog. They concluded that transcellular back diffusion of creatinine takes place. Levinsky and Berliner (1959)



a b

age area

Fig 2b Results based on the fractional *creatinine* and protein concentrations. Symbols as in Fig 2a

present evidence for back diffusion of creatinine from the ureter and suggest that this process is one of the reasons why a ratio below 1 00 is obtained An average ratio of 0 94 has been found in the present work

In microinjection experiments in the rat Bauman *et al* (1965), Maude *et al* (1965) and Gottschalk, Morel and Mylle (1965) have shown that inulin is not reabsorbed in the tubules

It is reasonable to presume that a creatinine to inulin clearance ratio below 1 00 is the result of back diffusion of the smaller creatinine molecule, and to rely on the assumption that inulin clearance is a measure of the glomerular filtration rate

The filtration fractions (FF_S and FF_D) The FF_S (S for superficial drainage area) averaged 0 34, using inulin as the indicator The average FF_D/FF_S calculated for various ranges of FF_S values was consistently below 1 00 A ratio of 0 85 may be used as a representative value (*cf* results and Fig 2a) FF_D/FF_S values below 1 00 were also obtained with creatinine as the indicator substance (Fig 2b)

The question may now be raised is the difference between the filtration fractions of the two areas explainable by distinct differences in the structure of the two areas

The FF of the plasma entering a glomerulus is a function of the colloid osmotic pressure of the plasma, and the hydrodynamic resistances of the afferent and efferent vessels and of the membrane of the glomerular capillaries also the rate of reabsorption of fluid by the proximal tubule is probably an important factor Bojesen 1954, Kruhoffer 1960)

When vascular by-passes of the glomeruli are present in an area the FF of the area will not be determined solely by the FF of the plasma entering the glomeruli, but the fraction of plasma traversing the

factor

Now, assuming that the FF of plasma entering the glomerular capillaries are identical in superficial and deep glomeruli but that some plasma by-passes the deep glomerular capillaries we may use the equation

$$F_D/(ARPF_D - ARPF_{BP}) = FF_S \quad (I)$$

where $ARPF_{BP}$ is the by-pass flow. The results of the experiments yielded (using the representative value of 0.85 for FF_D/FF_S)

$$F_D/ARPF_D \approx 0.85\ FF_S \quad (II)$$

II divided by I gives

$$ARPF_{BP}/ARPF_D - 1 - 0.85 = 0.15$$

Thus the by-pass flow should constitute a fraction of 0.15 of the plasma flow supplying the deep area. This is approximately 15   per cent = 6 per cent of the plasma flow supplying the whole kidney as the ratio $ARPF_D/ARPF_S$ is about   and therefore $ARPF_D/(ARPF_D + ARPF_S)$ —   The figure   is derived from the two facts 1) that the fractional protein concentration (V_P/A_P) of the superficial and deep drainage plasma averages 1.09 and 0.89 (Nissen 1965) and 2) that the mixing of these plasma streams must yield plasma (in the renal vein) with a fractional protein concentration very near to 1.00. The ratio between the two venous plasma flows D S is thus 1.09   1.00)   1.00—0.89) and the ratio between the arterial flows 1.09—1.00   0.89   1.00—0.89) 1.09 —

Various by-pass vessels situated outside the renal corpuscles of the deep cortex have been described these are the coiled arteries of the renal sinus the arteriolae rectae verae and the Isaacs Ludwig arterioles. The first vessels are aglomerular branches from the renal interlobar or arcuate arteries which partly end in the capillary plexus of the calyceine mucosa (v. Kugelgen and Passarge 1960) and partly supplement the vasa recta system supplying blood to the papillary zone (Baker 1959. The arteriolae rectae verae are aglomerular types of arteriolae rectae spuriae the latter forming the principal supply to the medulla. The Isaacs Ludwig arterioles are branches of normal afferent arterioles ending in the peritubular capillary plexus or as arteriolae rectae verae. For details the reader is referred to Selkurt (1963) v. Kugelgen *et al.* 1959) and Smith (1951). However these three kinds of aglomerular vessels of the normal kidney are rare so rare that it is difficult to imagine that about 6 per cent of the renal plasma flow passes through them.

Are there then — inside the corpuscles of the deep cortex — by-pass vessels of greater capacity? the works of Zlabek (1957) Ljungqvist and Lagergren (1962) and Granberg Lagergren and Ljungqvist (1964) offer information on this subject. Zlabek (1957) made polymethylmetacrylate casts of the vessels of 3 human kidneys ages 10, 18 and 38 years. Juxtamedullary glomeruli were defined as those possessing an arteriola recta spuria. Out of 62 normal (non degenerated) juxtamedullary glomeruli 56 possessed a wide vas efferens (of about the same width as the vas afferens. In 26 out of these 56 glomeruli and in none of the remaining a wide "short-circuit between the afferent and the efferent arteriole was clearly visible on the surface of the glomeruli. In the remaining 30 glomeruli a short circuit could not be observed

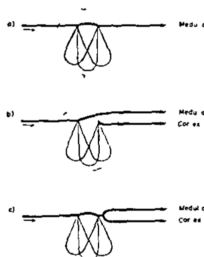


Fig 3 Sketch of proposed anatomy of juxtamedullary glomeruli
a) Juxtamedullary glomerulus with Zlabek s short circuit and one efferent arteriole
b) Juxtamedullary glomerulus with two efferent arterioles The blood to the medulla passes the short

low

by direct inspection, but in two of these a short circuit was observed on closer examination, the author believes that all juxtamedullary glomeruli with a wide vas efferens possess this short circuit placed superficially or deeply in the tuft The observation, that the efferent arterioles of the juxtamedullary glomeruli are wider than those of the rest of the glomeruli, was already made by Trueta *et al* (1947) Christensen (1952) could not affirm the observation however it was reaffirmed by Ljungqvist and Lagergren (1962) and Zlabek (1957)

Ljungqvist and Lagergren (1962) investigated the anatomy of the renal vessels by stereo-micro-angiographic and histologic techniques They found that degeneration of the juxtamedullary glomeruli owing to age produces atrophy of only the capillary tuft while the continuity between the afferent and efferent arterioles is retained and an aglomerular arteriole with an arteriola recta vera is formed In partly degenerated, juxtamedullary glomeruli the short circuit may be seen distinctly Degeneration of other glomeruli, however involves atrophy of the efferent vessel the glomerular tuft and the afferent vessel, in one stage of the process the latter is seen as a thin, blindly ending twig Ljungqvist and Lagergren (1962) believe that the differences between the degeneration forms are caused by the normally occurring short circuits described by Zlabek (1957)

Zlabek s (1957) short circuits, the arteriolae rectae verae and the coiled arteries supply the medulla These vessels would probably be adequate anatomical counterparts to the low FF_{D}/FF_{S}, since a plasma flow through them of approximately 6 per cent of the total renal plasma flow would not — a priori — be unreasonable A medullary flow of 6—8 per cent has been found in dogs by other authors Deetjen, Brechtelsbauer and Kramer 1964)

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Acta physiol. scand 1966. 68. 286—294

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The Relation between Reabsorption Rate and Filtration Rate in the Superficial and Deep Venous Drainage Area of the Cat Kidney

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Received 15 April 1966

Abstract

NISSEN, O I *The relation between reabsorption rate and filtration rate in the superficial and deep venous drainage area of the cat kidney* Acta physiol scand 1966 68 286—294

In 23 expts in cats the plasma concentrations of protein inulin and glucose have been measured in blood collected simultaneously from an artery and from subcapsular and deep veins of the kidney For each venous drainage area it is possible to calculate the ratio reabsorption rate filtration [illegible]

complicated pattern emerges for the drainage of the reabsorbate from different tubule segments to the vein systems

In a previous paper (Nissen 1966) the author presented a method for the determination of the filtration fractions of the plasma supplying the two venous drainage areas of the cat kidney The calculations were for each area based upon the concentrations of inulin and protein in the venous plasma relative to those of the arterial plasma

In the present paper a formula is given that expresses the rate of tubular reabsorption as a fraction of the glomerular ultrafiltration in the area Another equation gives the ratio reabsorbed to filtered amount of a substance in an area The renal handling of glucose is studied on this basis

The drainage of the reabsorbed fluid from different parts of the nephrons to the two vein systems will be considered in some detail

Theory

Calculation of the ratio reabsorption rate/filtration rate in a drainage area. The filtration fraction (FF) of the plasma supplying a drainage area is calculated as (Nissen 1966)

$$FF = \frac{1}{f_W} \cdot \frac{V_P/A_P - V_I/A_I}{V_P/A_P} \quad (1)$$

A_P is the concentration of protein in arterial plasma and V_P the concentration in venous plasma from the area in question. A_I and V_I are the corresponding concentrations of inulin. f_W (= 1.05) is a factor correcting plasma concentrations to concentrations in plasma water.

As the lymph flow is very small in comparison with the plasma flow, the venous plasma flow from an area (VRPF) must be equal to the rate of plasma flowing into the area (ARPF) minus the filtration rate (F) and plus the reabsorption rate (R) from the tubules of the area. Therefore

$$ARPF - F + R = VRPF$$

$$\text{or } 1 - F/ARPF + R/ARPF = VRPF/ARPF \quad (2)$$

F/ARPF is the filtration fraction (FF), R/ARPF = RF is the ratio between the rate of fluid reabsorption in the area and its arterial plasma flow.

As the glomerular capillaries are practically proteintight, we get

$$ARPF \cdot A_P = VRPF \cdot V_P \text{ or } VRPF/ARPF = A_P/V_P \quad (3)$$

Inserting 1 and 3 in 2 and isolating RF yields

$$RF = \frac{1}{f_W} \cdot \frac{f_W + V_P/A_P\,(1 - f_W) - V_I/A_I}{V_P/A_P} \quad (4)$$

$(1 - f_W)$ amounts to −0.05, and V_P/A_P is in the range of 0.85 to 1.15. The rest of the numerator $(f_W - V_I/A_I)$ generally has a magnitude of about 0.30. From this it follows that only a small percentual error of RF will be introduced if V_P/A_P is substituted by 1 in the numerator.

The simplified formula is

$$RF = \frac{1}{f_W} \cdot \frac{1 - V_I/A_I}{V_P/A_P} \quad (5)$$

The ratio between the RF and FF of an area equals the ratio between reabsorption rate and filtration rate in the area

$$RF/FF = R/F = \frac{1 - V_I/A_I}{V_P/A_P - V_I/A_I} \quad (6)$$

Calculation of the ratio reabsorbed amount/filtered amount of a freely filtrable non-electrolyte. The amount of the substance G (for glucose) carried to the drainage area by the arterial plasma, is $ARPF \cdot A_G$. The filtered amount is $F \cdot f_W \cdot A_G$ and the reabsorbed amount $R \cdot R_G$, where R_G is the average concentration of glucose in the reabsorbate. The amount drained away from the area is $VRPF \cdot V_G$. Provided formation and conversion of glucose in the area is negligible

$$ARPF \cdot A_G - F \cdot f_W \cdot A_G + R \cdot R_G = VRPF \cdot V_G$$

Isolating the unknown (reabsorbed amount/filtered amount) and inserting formula 1 and 3 yields:

$$\frac{R \cdot R_G}{F \cdot f_w \cdot A_G} = \frac{(VRPF/ARPF) \cdot (V_G/A_G) + (F/ARPF) \cdot f_w - 1}{(F/ARPF) \cdot f_w}$$

$$\text{or } \frac{R \cdot R_G}{F \cdot f_w \cdot A_G} = \frac{V_G/A_G - V_I/A_I}{V_P/A_P - V_I/A_I} \tag{7}$$

Discussion The simplification of formula 4 introduces a systematic error in formula 5 and 6 In the superficial venous plasma the fractional concentration of protein (V_P/A_P) and of inulin (V_I/A_I) amounts to about 1 10 and 0 70, respectively Therefore the RF of the superficial area is overestimated by 1—2 per cent by the simplification Conversely, when the deep venous plasma has a fractional protein and inulin concentration of about 0 90 and 0 65, the RF will be underestimated by 1(—2) per cent

However, an experimental fact tends to compensate for these errors In the superficial venous plasma the concentration of sodium with matching anions is 1(—2) per cent less than in arterial [illegible]

cent

The "simplification error" and the "osmotic error" partly cancel each other reducing the systematic error of the RF to 0 5—1 per cent (Neither formula 1 nor 7 is affected by the two errors because the simplification is not introduced and because the osmotic error changes the numerator and the denominator by the same factor since the red cell membrane in the cat is impermeable for protein inulin and glucose (Kozava 1914)

For a further discussion of methodological errors, see Nissen 1966

Method

The [illegible] reported in this study originate from a series of experiments partly described in two [illegible] in Nissen [illegible]

In addition to inulin in 0 9 per cent NaCl creatinine or glucose was also infused i v in some experiments *In other experiments the animal was loaded with p-aminohippurate sodium phosphate* or phloridzin The results from a few periods in which an osmotic diuresis exceeding 0 2 ml min from the experimental kidney was provoked are excluded The subject of osmotic diuresis will be treated in a subsequent paper

Results

The fractional concentrations from a single experiment of inulin, protein and glucose in blood collected from superficial and deep veins are given in Fig 1 Below the abscissa the results are tabulated A rise (29—51 mg per cent) in the level of the plasma inulin concentration was produced between period 3 and 4 R/F (formula 6 in "theory") refers to the ratio between distances on the graph, e g for period one R_3/F_5 equals a/b The fractional concentrations of inulin and protein in the superficial drainage plasma were higher than the respective fractional concentrations in the deep plasma FF_S was greater than FF_D (Nissen 1966) Furthermore RF_S FF_S was always below 1 and RF_D/FF_D above 1 This fact together with the observation

		1	2	3	4	5	6	7
$\frac{V_P}{A_P}$	S (superficial)	1.126	1.119	1.080	1.072	1.064	1.078	1.079
	D (deep venous)	0.913	0.923	0.807	0.936	0.929	0.939	0.913
$\frac{V_I}{A_I}$	S	0.778	0.742	0.764	0.776	0.776	0.762	0.766
	D	0.637	0.660	0.708	0.728	0.733	0.726	0.707
$\frac{V_G}{A_G}$	S	1.173	1.125	1.111	1.075	1.090	1.101	1.105
	D	0.885	0.890	0.910	0.912	0.908	0.916	0.910
FF	S	0.36	0.34	0.29	0.28	0.27	0.29	0.31
	D	0.30	0.28	0.22	0.22	0.21	0.23	0.23
$\frac{FF_D}{FF_S}$		0.83	0.84	0.76	0.80	0.77	0.78	0.75
$\frac{RF}{FF}$	S	0.63	0.76	0.76	0.75	0.77	0.75	0.77
	D	1.32	1.30	1.48	1.71	1.37	1.29	1.43
$\frac{reabs\ G}{filtr\ G}$	S	1.11	1.02	1.09	1.01	1.09	1.07	1.08
	D	0.90	0.88	1.01	0.90	0.90	0.89	1.00

Fig 1 Fractional concentrations for a single experiment of inulin protein and glucose in blood collected from superficial and deep veins of the kidney *Abscissa* Period number *Ordinate* Fractional concentrations of protein (P) of inulin (I) and of glucose (G in the drainage plasma indicates the deep plasma ● the superficial plasma a/b is equal to RFs/FFs = Rs Fs

Periods 1—3 were carried out without pauses Between periods 3 and 4 half an hour elapsed where the level of plasma inulin concentration was raised from 29 mg per cent to 54 mg per cent Periods 4—7 were again carried out in succession

The results of the experiments are tabulated below

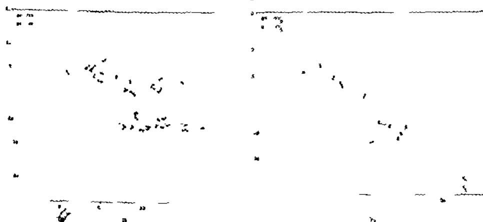


Fig 2a Results based on the fractional ul and prote n c en rat ons ● ind tes b its abscissa the FFs and by its ordinate RFs FFs n l cates by ts abs ssa and or l nate the quantit es FFD and RFD FFD Each period of an exp r men elds a po t and a cross The ord nate is logar thmic The symbols are explained in the appendix

Fig 2b Results based on the fra nal reat n ne and prote n c n en rat ns Sym bols as in Fig 2a

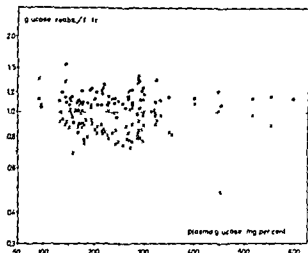


Fig. 3. *Abscissa:* Concentration of glucose in arterial plasma. *Ordinate:* ● represents the ratio reabsorbed amount/filtered amount of glucose in the superficial drainage area. × represents corresponding ratios in the deep area.

that the ratio reabsorbed amount to filtered amount of glucose is generally above 1 for the superficial area and below 1 for the deep area, will be discussed below.

The rise in the plasma inulin level did not produce any changes in the results.

RF/FF = R/F. In Fig. 2a all the RF/FF values are given using inulin as the indicator substance (23 expts., 78 periods). Essentially the same results were obtained using creatinine — but fewer periods (46) were made with this substance (10 expts. — Fig. 2b).

With inulin as the indicator the average values for RF_S/FF_S in the FF_S ranges 0.1—0.2, 0.2—0.3, 0.3—0.4 and 0.4—0.5 were 0.7, 0.79, 0.79 and 0.77; the standard error (S.E.) of the last three means was 0.01. The average values for RF_D/FF_D in the corresponding FF_D ranges were 1.5, 1.37, 1.23 and 1.33. The S.E. of the last three means were 0.03, 0.02 and 0.03. All the RF_S/FF_S and RF_D/FF_D ratios where S.E. is stated are below 1 and above 1 for $p < 0.001$ (Student's t).

Glucose. In 76 periods of 19 expts. the glucose threshold was not exceeded, as the ratio excreted/filtered amount of glucose was below 0.01.

In these periods the ratio reabsorbed/filtered amount for the superficial area averaged 1.13, S.E. = 0.01. For the deep area the average value was 0.87, S.E. = 0.01. The two means were different from 1.00, $p < 0.001$ (Fig. 3).

Discussion

Two major findings will be discussed. Firstly, that the reabsorption rate is lower than the filtration rate in the superficial drainage area and that the opposite applies to the deep area (Fig. 1 and 2). Secondly, the paradoxical finding that the ratio reabsorbed/filtered amount of glucose is above 1 in the superficial area and below 1 in the deep area.

As an introduction a short summary of *renal anatomy* will be given. The borderline separating the two drainage areas is indicated in Fig. 4 by the upper thin horizontal

line The border of the medulla is marked by a thick line All nephrons in the kidney are symbolized by two nephrons, the corpusculum of one lying in the superficial cortex, and that of the other in the deep cortex The pars convoluta of the proximal tubule is horizontally depicted (the "loop" of the proximal tubule of the deep glomerulus is referred to later in the paper) From the end of the convoluted tubule the pars recta stretches to the medulla The pars recta becomes the thin limb of the loop of Henle at the transition between the outer and inner stripe of the outer medullary zone

All the loops of the cat kidney are long, viz they reach into the inner medulla Where the latter borders the outer medulla the distal thin limb continues in the thick limb of the loop of Henle The thick limp passes out into the cortex and after running a course close to its corpuscle, it is gradually converted into an initial and proper collecting tubule The majority of these transitions are situated in the most superficial part of the cortex — this applies even to nephrons with deeply lying corpuscles Thus most of the nephrons possess not only a "medullary loop but also a "cortex loop" From an anatomical aspect considerable importance has been attached to the description of these cortex loops in different species (Peter 1927, v Mollendorff 1930, Sperber 1944)

From this provisional scheme it is reasonable to assume that the fluid ultrafiltered from the blood passing the superficial area is partly reabsorbed into this blood and partly (in the loops of Henle and the collecting tubules) to that traversing the deep area, further that the fluid ultrafiltered from the deep blood is reabsorbed partly into the deep blood (in the proximal tubules, loops of Henle, and collecting tubules) and partly into the superficial blood (by the cortex loops and the upper part of the collecting tubules) It may be presumed that the fluid volume ultrafiltered superficially and reabsorbed profoundly exceeds the volume ultrafiltered profoundly and reabsorbed superficially, as the proximal tubules constitute a considerable part of the "medullary loops, the fewer and shorter cortex loops consist of distal tubules and collecting tubules This view is consistent with the finding of a R F ratio (cf appendix) below 1 in the superficial area and above 1 in the deep area

However, the model as described so far cannot explain the fact that more *glucose* is reabsorbed than filtered in the superficial area, and vice versa in the deep area (Fig 1 and 3) viz if the view that glucose is reabsorbed merely in the proximal tubules holds good then the ratio reabsorbed amount filtered amount of glucose in the superficial area should be 1 or less The value 1 would occur if the reabsorption of the glucose filtered in the superficial area was completed in those parts of the proximal tubules, which are surrounded by blood drained by the superficial veins In this case the ratio reabsorbed amount filtered amount of glucose in the deep area would adopt its minimal value which is also 1 as long as T_{max} glucose is not exceeded)

It seems unlikely that a conversion or synthesis of glucose quantitatively is responsible for the considerable deviations of the ratio from 1 From a qualitative point of view such an explanation seems equally unlikely because it would be necessary

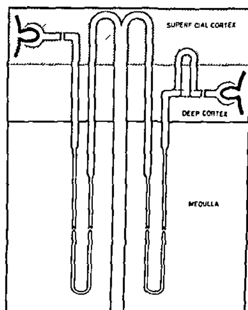


Fig 4 Model of the nephron population The nephrons are represented by one symbolizing those originating in the superficial drainage area another those originating in the deep area

to assume that a conversion took place in one area (the deep one), while a synthesis occurred simultaneously in the other The key to the problem may lie in the localisation of the tubules belonging to glomeruli situated along the border, separating the *two drainage areas* Anatomists *Peter 1927, v Mollendorff 1930) often emphasize* that the glomerulus of the mammalian kidney is situated profoundly (centrally) to the attached ball of the proximal tubule From the descriptions and drawings of these balls of the cat (Peter 1927) it may be deduced that the proximal tubules of the middle part of the cortex often have their loops lying 1 0—1 5 mm in the superficial direction from the attached corpusculum The total thickness of cortex is 4—6 mm

Now it can be seen that a certain number of bordernephrons are filled with a fluid ultrafiltered from the plasma that supplies the deep drainage area while the proximal tubules of these nephrons are partly bathed in plasma, that is drained by the superficial veins The existence of these nephrons is indicated in Fig 4 by a loop *of the proximal* tubule extending into the superficial area

A ratio between reabsorbed and filtered amounts of glucose above 1 for the superficial area and below 1 for the deep drainage area may now be explained viz it results from a reabsorption into the superficial plasma of some of the glucose ultra filtered from the deep plasma in the glomeruli of the border-nephrons If any glucose was reabsorbed in the cortex loop, these formations might also account — at least partially — for the deviations of the ratio from 1

The intratubular transit of glucose across the border averages 13 per cent of the amount of glucose filtered in the superficial area (reabs G filtr G = 1 13 The great majority of these proximal loops of the borderline were probable saturated in

respect to glucose transport as the plasma glucose concentrations were high, although they did not exceed the plasma threshold (Fig. 3). Further it is probable that some proportionality exists between the glucose transport capacity and the rate of fluid reabsorption of the proximal tubule mass. Accepting this it may be suggested that a reabsorbate volume amounting to roughly 10 per cent of the superficial ultrafiltrate is transported from the deep to the superficial area by the border-nephrons.

This estimate makes it possible to give a rough quantitative description of the handling of the volume, ultrafiltered in the superficial drainage area.

Assuming the ratio R_S/F_S to be 0.78 (p. 5), per 100 ml ultrafiltrate of the superficial area, 78 ml are reabsorbed by the tubules of this area. About 10 ml originate from the proximal loops of the border nephrons and thus they are ultrafiltered by the deep glomeruli. The rest, 68 ml, are proximal reabsorbate (ultrafiltered superficially) plus distal reabsorbate from the cortex loops and the collecting ducts situated in the superficial area (Fig. 4). Presuming the latter reabsorbate volume to be 5 ml (cf. for example Kruhøffer 1960, p. 250) it will be seen that approximately 68 — 5 = 63 ml of the 100 ml filtered are reabsorbed by the proximal tubules of the superficial area. The remaining 37 ml, are reabsorbed by the loops of Henle, by the rest of the distal tubules, and by the collecting ducts, a minor part is excreted as urine.

On the kidney surface 55 to 60 per cent of the proximal tubule length was accessible in the micropuncture experiments of Walker *et al.* (1941) in the rat and Clapp, Watson and Berliner 1963 in the dog. From these works and from that of Peter (1927) it may be worked out that about 3/4 of the length of the proximal tubules of superficial origin lie in the superficial drainage area, assuming a borderline in the middle of cortex. If the view set forth by Walker *et al.* (1941) that the reabsorbed amount of fluid per length unit of proximal tubules is constant throughout the tubules, then 100 — (63 · 4/3) = 16 ml would be handled by the distal tubules.

Appendix

Definition of Symbols

D and S – Subscripts referring to deep and superficial renal venous drainage area
ARPF = Arterial renal plasma flow supplying a venous drainage area
VRPF Venous renal plasma flow leaving a venous drainage area
F = Volume ultrafiltered per time unit in a venous drainage area
R = Volume reabsorbed by the tubules per time unit [illegible] a venous drainage area
FF = F/ARPF = filtration fraction in a drainage area
AX = The plasma concentration of a substance X in arterial blood
VX = The plasma concentration of a substance X in venous blood from a drainage area
VX/AX = The fractional concentration of X
I P G = Subscripts referring to inulin, p[illegible] and glucose
fw – 1.0[illegible] is a factor which correc[illegible] plasma [illegible] entra[illegible] of a substance [illegible] the concentration of the substance in the plas[illegible] water

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Acta physiol. scand 1966. 68. 295—303

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The Action of Adrenaline in Cardiac Muscle II.

Effect on Oxygen Consumption in the Asystolic Perfused Rat Heart

By

ANTON HAUGE and IVAR OYE

Received 23 April 1966

Abstract

HAUGE A and I OYE *The action of adrenaline in cardiac muscle II Effect on oxygen consumption in the asystolic perfused rat heart* Acta physiol scand 1966 68 295—303

Oxygen cons[illegible]

which directly or indirectly are stimulated by adrenaline

Adrenaline increases the oxygen consumption of cardiac muscle (Barcroft and Dixon 1907) The increase can only partially be accounted for by an increased stroke work and the term "oxygen waste" has been introduced to describe the excess increase in oxygen uptake (Raab 1962) The 'oxygen waste' might reflect either an increase in contractile activity which does not result in increased pump work ("internal work") or a direct effect of adrenaline on cardiac metabolism, or both An increase in cardiac oxygen consumption after adrenaline during experimental asystolia in the perfused rat heart has been described by Challoner and Steinberg (1965) and by Hauge and Oye (1966) In the present work the calorigenic effect of adrenaline has been studied under various types of experimental asystolia and an effort has been made to elucidate the mechanism involved in this adrenergic response

As working hypotheses the following possible mechanisms were considered 1 If the supply of oxygen or oxidizable substrates is ratelimiting for oxidative phosphorylation, the calorigenic effect of adrenaline might be secondary to either an increased coronary flow or i[illegible] mobilization of endogenous substrates (lipolytic and glycogenolytic acti

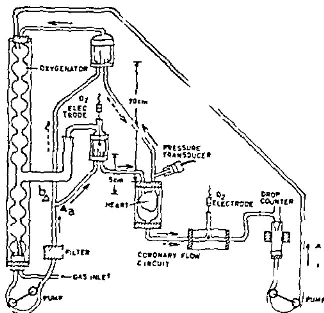


Fig 1 *Perfusion arrangement* The perfusion method previously described (Øye, 1965) was modified to allow continuous recording of coronary flow and pO_2 The coronary effluent is ejected through the open pulmonary artery into the closed chamber in which the heart is mounted and flows through a rigid tube (surrounded by a waterjacket) into which the oxygen microelectrode is closely fitted through a side tube Finally the coronary effluent flows through a photo-electric drop counter from which it is pumped back to the top of the oxygenator Arrows show flow direction during anterograde perfusion (tube to upper reservoir clamped at and broken arrows show flow direction during retrograde perfusion (tube to lower reservoir clamped at ▲

2 If the oxidative phosphorylation is limited by the rate of energy utilization the calorigenic effect of adrenaline might be secondary to either an increased utilization of ATP, or to an 'uncoupling' of the oxidative phosphorylation process

Experiments were designed to examine the main determinants for cardiac oxygen consumption during asystolia, and possible target systems through which adrenaline might influence these determinants are discussed

Methods

Rats of both sexes about 300 g local strain, originally Wistar were used The hearts were excised under ether anesthesia and perfused by the method developed by Morgan (1961 as previously described Øye 1965 This perfusion technique involves cannulation of the left atrium as well as the aorta The left atrium is connected to an overflow reservoir 5 cm above the level of the heart and the aortic cannula is connected to a similar reservoir 75 cm above the heart When the tube leading to the left atrium is open perfusate will enter the heart through the left atrium and is pumped by the heart to the upper reservoir thus allowing the heart to perform a physiological type of work *in vitro* (anterograde perfusion When the tube leading to the left atrium is closed the perfusate will flow retrogradely through the aorta and coronary vascular bed as in a classical Langendorff type of perfusion retrograde perfusion In both cases the coronary effluent is expelled through the right ventricle into the closed chamber where the heart is mounted In order to measure cardiac oxygen usage the perfusion circuit was modified as shown in Fig 1 allowing continuous recording of coronary pO_2 with a Clark oxygen micro electrode and a Beckman Physiological Gas Analyzer Model 160 Coronary flow was measured with a Palmer phototransistor drop counter Inflow pO_2 was measured by mounting the oxygen electrode in the reservoir connected to the left atrium Oxygen consumption was calculated from the arterio-venous pO_2 difference and the oxygen solubility constant (Umbreit *et al* 1957 The perfusate was a modified Krebs Ringer bicarbonate buffer (Øye 1965) gassed continuously with 95% oxygen and 5% carbon dioxide The buffer contained 1 per cent albumin bovine fraction V Sigma and 10 mM glucose Other additions are stated under results The total volume of perfusate was 40 ml and the temperature was kept constant at 32° C.

Lactate was measured enzymatically with lactate dehydrogenase and NAD according to H[illegible] (1963, Glycerol was measured according to Burton 1957

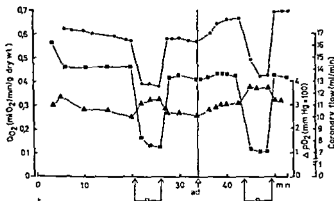


Fig 2 *Oxygen consumption* (Q_{O_2} — ● —) *coronary flow* (— ■ —) *and arteriovenous* pO_2 *difference* ($\triangle pO_2$ — ▲ —) *during anterograde and retrograde perfusion*

After 20 min of anterograde perfusion flow direction was changed to retrograde perfusion (no pump work done by the heart) for five min (R) At 35 min 3 μg adrenaline was added As shown by the figure the increase in Q_{O_2} in the subsequent periods of anterograde and retrograde (R) perfusion is mainly due to an increase in $\triangle pO_2$

Adrenaline bitartrate (Rhone Poulenc) was dissolved in 0 9 per cent sodium chloride containing 1 mM EDTA and stored frozen The adrenergic receptor blocking agents used were phentolamine (Regitin® Ciba), propranolol (Inderal® I C I) and dichlorophenylisopropylaminoethanol (DCI Aldrich Chemical Cp Inc) The procainamide solution used was the commercial preparation Pronestyl®, Squibb

Results

Oxygen consumption in the working and arrested heart

The average oxygen consumption of the isolated, working rat heart at 32 C, under the present experimental conditions, was 0 56 ml min per g dry weight or about 13 ml/min per 100 g wet weight, (the wet weight/dry weight ratio in hearts perfused for 30 min being about 4 5) Fig 2 illustrates the typical change in oxygen consumption (Q_{O_2}), coronary flow and arterio-venous pO_2 differences ($\triangle pO_2$) when the flow direction of the perfusate was changed from anterograde perfusion to retrograde perfusion, before and after the addition of adrenaline During the period of anterograde perfusion the left ventricle contracted against a diastolic pressure of 75 cm perfusate During the period of retrograde perfusion the perfusate was flushed through the aorta and the coronary vascular bed The left ventricle was not filled during the diastole, and no external work (pumping was done as shown by no increase in the recorded aortic pressure during systole Usually no change in rate or rhythm occurred when the flow direction was shifted from anterograde to retrograde The mean value for oxygen consumption during retrograde perfusion was 70 per cent of the oxygen consumption during work

A further decrease in oxygen consumption occurred when the hearts were made asystolic by increasing the extracellular potassium concentration by adding sodium citrate or by the use of procainamide The oxygen consumption during asystolia

TABLE I Oxygen consumption in perfused hearts during work and in the non working state. The hearts were perfused by anterograde perfusion as described under methods for 15—20 min in order to obtain steady values for coronary flow and pO_2. Asystole was induced by *exchanging 10 ml of the perfusate with 10 ml of a similar medium in which potassium had been substituted for sodium giving a final concentration of 34 meq/l of potassium (group I) or by the addition of 2 ml 1 M sodium citrate to give a final concentration of 48 mM* (group II) or by adding 0.2 g procainamide to make 15 mg/ml (group III). During asystole the hearts were perfused retrogradely through the aorta. In group IV retrograde perfusion was established by clamping the tube leading to the left atrium

Experimental group	Conditions during experimental period	Oxygen consumption	
		Control period	Experimental period
		O_2 in ml per min per g dry weight	
I	Arrested with potassium (17)	0.50 ± 0.04	0.16 ± 0.02
II	Arrested with pronestyl[R] (8)	0.5[illegible] ± 0.04	0.27 ± 0.04
III	Arrested with citrate (8)	0.5[illegible] ± 0.05	0.38 ± 0.02
IV	Reversion of flow to retrograde perfusion (4)	0.58 ± 0.0[illegible]	0.41 ± 0.02

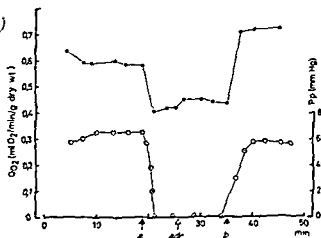


Fig. 3 Oxygen consumption ● and pulse pressure ○ before and after the addition of sodium citrate (final concentration 30 mM) at 20 min (a). Adrenaline (3 μg) was added at 25 min. At 35 min (b) 0.150 M $CaCl_2$ was infused until the pulse pressure reached normal levels. After adrenaline Q_{O_2} is increased in the asystolic as well as in the working state

depended on the method used to arrest the heart (Table I). In hearts arrested with citrate addition of $CaCl_2$ restored contractions and brought oxygen consumption to control levels (Fig. 3). In hearts arrested with potassium or procainamide contractions and oxygen consumption were restored on renewal of the perfusate.

The possibility that coronary flow *per se* is a determinant of Q_{O_2} during asystolia was tested by adding adenosine which is known to reduce resistance to flow through the coronary vascular bed (Berne 1964). 1 mmole/l of this nucleoside increased flow rate in hearts arrested with potassium to an extent which resulted in a raised

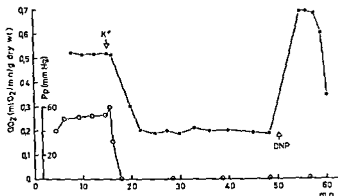


Fig 4 *Effect of dinitrophenol*
Oxygen consumption (—●—) and pulse pressure (—⊙—) in a heart arrested with high concentrations of potassium At 16 min infusion of the potassium buffer was started At 50 min dinitrophenol (final concentration 1 mM) was added to the perfusate

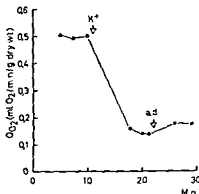


Fig 5 *A typical experiment showing the effect of adrenaline on oxygen consumption during asystolia*
The heart was arrested at 15 min by increasing the potassium concentration as described under "methods At 24 min 10 μg adrenaline was added to the perfusate

Qo_2 despite decreased arteriovenous pO_2 difference However, when the flow rate was kept constant by mechanical regulation of the perfusion rate, adenosine was without effect on oxygen consumption The nucleotides 5-AMP and cyclic 3 5-AMP had effects similar to adenosine

Additional supply of substrates (5 mmols each of pyruvate, lactate and beta-hydroxybutyrate) was without significant effect on oxygen consumption of the arrested hearts Since the albumin used contained a substantial amount of fatty acids, the effect of substrate addition to hearts perfused for some time in the complete absence of substrates was not tested

The rapid adjustment of Qo_2 to changes in the physical activity indicates that energy utilization is a main determinant of Qo_2 in the isolated perfused heart Restriction of oxygen consumption by the rate of oxidative phosphorylation persists also in the asystolic state as demonstrated by the dramatic effect of agents which uncouple oxidative phosphorylation Fig 4 shows a typical experiment with a rela-

rat heart, it appears unlikely that the rapid calorigenic response observed in our experiments are secondary to the glycolytic or lipolytic effects. This does not exclude the possibility than an increased amount of free fatty acids contributes substantially to the calorigenic effects of adrenaline during longer periods of perfusion.

A role of coronary flow as a determinant of oxygen consumption during experimental asystolia was revealed by the use of adenosine and the related nucleotides 5'-AMP and 3',5'-AMP. However, the calorigenic effect of adrenaline did not depend on an increase in coronary flow and was found to be present even when adrenaline caused a marked decrease in flow.

If cardiac metabolism is interfered with, loss of the functional properties rapidly occurs (Bing 1965). The reversibility of the present preparation from the asystolic to the working state indicates that the essential metabolic processes were maintained during the period of asystolia and it is reasonable to assume that they were more or less subject to the same basic control mechanism as in the working heart. The effect of 2,4-dinitrophenol shows that the respiratory control through oxidative phosphorylation persisted. Under such conditions enhanced energy expenditure leads to increased oxygen consumption by increasing the amount of ADP and P_i available for oxidative phosphorylation. Adrenaline is known to stimulate biochemical reactions that use ATP in non-working tissues. A well known example is the phosphorylation of phosphorylase b to form phosphorylase a (Krebs et al. 1964). In this process 4 moles of ATP are used to convert 2 moles of phosphorylase b to 1 mole of phosphorylase a, and 4 moles of ADP are formed. Phosphorylase a is rapidly dephosphorylated by a phosphatase to form phosphorylase b and P_i, the net result of the cycle being the formation of 4 ADP and 4 P_i. Glycogen synthetase (Rosell-Perez and Larner, 1964) and possibly also lipase (Rizack 1963) are subject to similar phosphorylation processes which appear to be stimulated by adrenaline. Adrenaline thus increases the energy expenditure not only in the contracting muscle but also in the asystolic heart. The inotropic and chronotropic effects in the beating heart, as well as the initiation of contractions during experimental asystolia, can be regarded as representing a direct or indirect stimulation of reactions which require energy. Increased ATP utilization appears to be a common denominator for the various expressions of adrenergic stimulation of cardiac muscle. This applies to the metabolic actions of adrenaline as well as to its effects on the physical activity of the muscle. It thus seems justified to explain at least part of the calorigenic effect of adrenaline during experimental asystolia as secondary to increased utilization of ATP by several separate reaction systems all of which contribute to the basal energy expenditure of the heart.

This work was supported by a grant from Nordisk Insulin Fond and from Det Norske Råd for Hjerte og Karsykdommer.

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Acta physiol. scand. 1966. 68. 304—315

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Importance of Tissue Pressure for the Fluid Equilibrium between the Vascular and Interstitial Compartments in the Small Intestine

By

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Received 25 April 1966

Abstract

WALLENTIN, I *Importance of tissue pressure for the fluid equilibrium between the vascular and interstitial compartments in the small intestine* Acta physiol scand 1966 *68*. 304 315

The effect of [illegible]
was studied o[illegible]
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to a shift of the Starling equilibrium, an indirect method was used consisting of stepwise raising and lowering the venous pressure During this procedure the rate of net fluid transfer upon a given venous pressure level could differ very much depending on whether this pressure level was reached from a lower or from a higher venous outflow pressure The transcapillary forces responsible for these differences of net fluid transfer amounted to often more than 10 mm Hg This means that some factor or factors involved in the Starling equilibrium must have changed by this amount It is suggested that increasing tissue pressure is the most important counterforce during outward filtration caused by a sudden rise of the venous outflow pressure Evidently the small intestine is quite well protected against edema in spite of a very big transcapillary filtration capacity

The relationship between intestinal blood flow resistance and capillary filtration coefficient (CFC) was described in a previous paper (Folkow, Lundgren and Wallentin 1963) The small intestine was shown to have a big capacity for fluid transport over the capillary membrane CFC, calculated for the mucosa portion, appeared to be in the magnitude of 1ml min / mm Hg / 100 g tissue) at maximal vasodilatation, a figure in the range of the glomerular filtration in the human kidneys The maximal blood flow in the mucosa was deduced to be about 500 ml min / 100 g tissue), *i.e.* some 10 times that of a skeletal muscle

The big capacity for capillary transfer in the intestine seems to be well suited for the specialized functions of the mucosa as regards secretion and absorption It implies, however, a potential danger that even slight increases in mean capillary pressure might rapidly cause an extensive intestinal edema

Fortunately, the control of the mean capillary pressure in the intestine seems to be extremely good

1 Differences in hydrostatic pressure in intestinal capillaries, depending on the position of the intestinal loops in relation to the heart, are counterbalanced by similar hydrostatic pressure differences in the surrounding abdominal contents, opposing the tendency for pooling of blood (Rushmer 1946) and also fluid losses owing to increased filtration

2 The autoregulation of blood flow for different arterial pressure levels is pronounced (Johnson 1960, 1964) and seems to be more adapted for regulation of capillary pressure than for nutritional blood flow (Johnson 1964)

3 Changes in vasoconstrictor tone, whether these changes are induced reflexly (Öberg 1964), or by stimulation of the sympathetic vasoconstrictor fibres (Folkow *et al* 1964 a, b), do not modify the transcapillary fluid balance in the intestine, indicating an unchanged mean capillary pressure In the skeletal muscle on the other hand, increased vasoconstrictor tone lowers the mean capillary pressure, which causes an often extensive absorption of fluid (Mellander 1960, Öberg 1964)

If, on the other hand, a primary rise in venous pressure occurs, active vascular adjustments have far less chance to counteract the influence of the venous pressure rise on the capillary pressure level There must be many situations in which venous pressure really becomes raised, as in portal hypertension due to liver cirrhosis, right heart insufficiency, subclinical and manifest strangulation ileus, etc Of course, it must be remembered that several other factors, besides the capillary pressure, determine the transcapillary fluid balance The well known Starling equilibrium implies that the mean hydrostatic capillary pressure minus tissue pressure equals plasma colloid osmotic pressure minus tissue colloid osmotic pressure when no net transcapillary transport occurs Therefore if mean capillary pressure is raised, it will cause an outward filtration, which theoretically produces a lowering of the colloid osmotic pressure in the interstitial fluid, an increase in the plasma colloid osmotic pressure and an increase in tissue pressure, all events which will counteract further filtration

It was for such reasons considered to be of interest to know what happens in the transcapillary filtration absorption exchange during prolonged increases in venous outflow pressure, and to discuss the relative importance of the different factors in the Starling equilibrium

Methods

The [illegible] ndgren and Wallentin (1963 and this [illegible]) cats weighing 2—4 [illegible] mented as required) [illegible] of the small intestine [illegible] After heparinization the superior mesenteric vein draining the intestinal segment and its lymph glands was cannulated and the venous outflow passed an optical drop recorder-ord nate-writer unit after which it was returned to the animal The intestine was autoperfused via a polyethylene tube from one carotid artery to the superior mesenteric artery The intestinal pedicle was divided so that the preparation was totally denervated Arterial blood pressure was measured from one femoral artery The venous

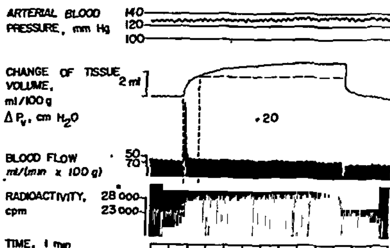


Fig 1 Cat 2 kg Effects of a prolonged period of raised venous outflow pressure on tissue volume and on the regional blood volume (the curve of radioactivity)

Note that the volume increase representing the outward filtration, is decreasing gradually, reaching a new isovolumetric state after about 8 min

outflow pressure measured with a saline manometer could be set at any desired level by adjusting the height of the tube end which drained the drop recorder The intestine, now only connected to

The temperature of the animals was maintained by an electric heating pad and an infrared lamp Great care was taken to maintain a constant temperature for the intestinal preparation in the pletysmograph

Intestinal lymph was collected in 3 cats A polyethylene catheter was inserted in one of the biggest lymph vessels in the pedicle and the lymph was collected for periods of 15 or 30 min at different venous outflow pressures

... processes from changes in the regional blood volume an

... background activity

20,000—40,000 cpm.

Results

To determine the capillary filtration coefficient (CFC) venous outflow pressure is raised for a relatively short period, 1 1/2—3 min This produces an initial rapid increase in the intestinal volume, due to venous distension, followed by a slower phase of increase, due to outward filtration The filtration slope is proportional to

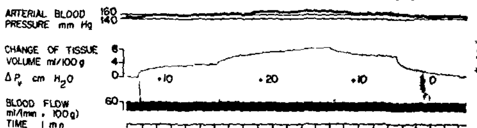
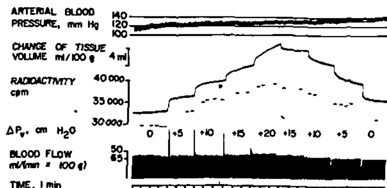


Fig 2 Cat 2 3 kg The venous outflow pressure is raised stepwise by 10 cm water and thereafter lowered again Note the tendency for the intestinal volume to level off towards an isovolumetric state both at raised and lowered venous pressure

the rise in mean capillary pressure and the capillary surface area open to flow and hence for filtration However, in the present investigation when the period of increased outflow pressure was prolonged, the filtration rate decreased gradually and often ceased more or less completely within 10—15 min This typical response can be seen in Fig 1 where the outflow pressure was raised by 20 cm water In this case the reactivity of the resistance vessels and precapillary sphincters was diminished by a constant intraarterial infusion of isopropyl noradrenaline (Pafadrin, AB Hässle) ca 0 3 μg/min The changes in radioactivity emitted from the Cr^{51} labelled erythrocytes indicate that the blood content in the preparation increased when the venous pressure was raised, first very rapidly and thereafter at a slower rate during 40—50 sec, indicating an obvious delayed compliance of the capacitance vessels During the rest of the period of raised venous pressure a fairly small and steady increase in radioactivity is seen In case this increase also corresponds to increasing blood volume it amounts to only 15—20 per cent of the simultaneous increase in tissue volume Another possibility is that it corresponds to small areas with very slow blood circulation, *e g* mesenterial fat or areas close to the ligated ends of the intestine The record of tissue volume change, on the other hand shows that the filtration is decreasing gradually during the whole period, reaching a new isovolumetric state after about 6—7 min This means that a rise of 20 cm water in venous pressure, corresponding to about 12 5 mm Hg rise in mean capillary pressure (Folkow, Lundgren and Wallentin 1963) and a consequent filtration, was gradually balanced by some counterforce Provided that no gradual shift in vascular tone changing the pre/postcapillary resistance ratio occurred, some of the other factors of the Starling equilibrium must have been changed This tendency to level off gradually was seen both when raising and lowering the venous pressure as illustrated in Fig 2 Here the outflow pressure was raised in steps of 10 cm water and then lowered again Thus, normally a new isovolumetric state was ultimately reached even when stepwise increases in capillary pressure were performed Sometimes however a small volume increment, about 5—10 per cent of the initial outward filtration continued even after a prolonged period [illegible] capillary pressure, possibly reflecting filtration from serosal and mucosal [illegible] the amount of which is dependent on the degree of hyperemia in [illegible]



volume

Observe that in case of no counterforces against the filtration each pressure level should cause the same amount of outward filtration independently whether this pressure level was preceded by a higher or lower venous outflow pressure

a filtration loss can, of course, *not be counterbalanced* by *e g increases in tissue pressure* or by dilution of interstitial proteins It should perhaps be mentioned here that cats with infections, especially intestinal ones, often developed distended, edematous intestines, and in these cases rapid and fairly stable volume increases occurred whenever venous outflow pressure was kept increased Cats, with evidence of such a pathophysiological disturbance of the transcapillary fluid exchange, could therefore not be utilized for the present purpose, where in the first hand the normal reactions were *to be studied*

Fig 3 shows what happens when the outflow pressure is increased in equal steps lasting about 3 min and then lowered successively again It is obvious from the *figure that any level of increased venous pressure causes filtration during the way* up, but an absorption of fluid during the way down The radioactivity levels show that the blood content after the period of delayed compliance (40—50 sec) is fairly constant at most pressure levels Where obvious shifts in blood volume occur, *e g* at 20 cm water, these changes are corrected for during the evaluation of the filtration slopes These corrections are, however, quite small The slopes at each venous pressure level, corresponding to filtration or absorption are plotted in Fig 4 and constitute, when connected, a characteristic loop, which was obtained every time the venous pressure was raised stepwise and thereafter lowered again, as in Fig 3 The increase of filtration between each step from + 5 to + 20 cm water is fairly constant, indicating that also *CFC is nearly constant during this period* The intersections with the abscissa denote zero slope, i e when no filtration or absorption occurs, and show that isovolumetric state can be reached at different levels of venous outflow *pressure, in this* case more than 15 cm H_2O apart, during this run of stepwise raising and lowering the venous pressure The vertical distance between

Fig 4 The slopes of filtration or absorption from Fig 3 are plotted and connected to make a loop On each level of venous outflow pressure two values are seen, one representing the outward filtration when the venous pressure is raised the other representing the absorption when the pressure is thereafter lowered Zero slope i e the isovolumetric state is reached at different levels more than 15 cm apart The vertical distance between the two parts of the loop represents the difference in net fluid transfer at each pressure level At + 10 cm water this difference amounts to 0 85 ml/(min × 100 g) When divided by the simultaneous CFC 0 064 ml/(min × mm Hg × 100 g) the corresponding change in the Starling equilibrium in this case 13 mm Hg is known

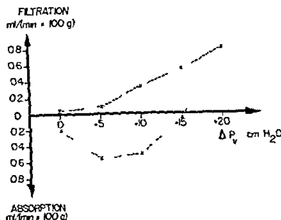
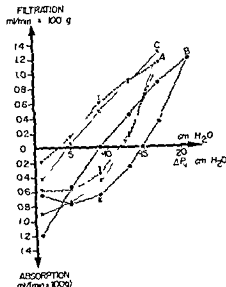


Fig 5 Each loop A B and C, are plotted similar to the loop in Fig 4 Loop A represents a control run of stepwise changing the venous outflow pressure as in Fig 3 Thereafter a prolonged outward filtration was caused by raising the venous pressure by 13 cm water during 7 min (Not shown in the figure) Loop B representing a run just after the venous pressure was lowered again shows that additional filling of the tissue spaces with filtrate displaces the loop to the right 20 min later when the fluid filtered during the long filtration period was absorbed again a new run (loop C) was almost identical with the control run loop A The



the two parts of the loop expresses the difference in the rate of net fluid transfer at the same level of venous outflow pressure When this difference is divided by the CFC obtained at the same level, the corresponding change in the Starling equilibrium which could explain this difference is known In Fig 4 the maximal difference in net fluid transfer is obtained at + 10 cm water and amounts to 0 85 ml (min 100 g tissue), CFC is calculated to 0 064 ml min mm Hg × 100 g tissue) This gives in this particular experiment a figure of about 13 mm Hg pressure difference in the Starling equilibrium The mean value of the maximal differences for 44 loops from 11 cats was 9 1 ± 2 33 SD mm Hg This must mean that either the tissue pressure has increased or/and the interstitial colloid osmotic pressure dro-

static capillary pressure has decreased by a corresponding amount Any of these factors alone, or in combination, can, of course, be responsible here There are no reasons to assume that the colloid osmotic pressure of the blood has changed to any significant extent (see below)

Intraarterial infusions of papaverine HCl, in doses sufficiently high to eliminate completely the tone and reactivity of the resistance vessels (1—3 5 mg/min) did not change these loops or diminish significantly the maximal difference mentioned above This proves that the mentioned events are not consequences of slow vascular adjustments causing capillary pressure changes, but reflect changes of either tissue pressure or/and interstitial colloid osmotic pressure

In Fig 5 each loop (A, B and C) represents a run of stepwise raising and lowering venous pressure as in Fig 3 and 4 After a control run (loop A), the venous pressure was raised with 13 cm water, producing an outward filtration, which after 7 min had decreased to about 15 per cent of the initial value (This long filtration period is not shown in the figure) After lowering the venous pressure again, new runs were performed, first immediately (loop B) and then 20 min later, when the fluid filtered just before loop B had been absorbed again (loop C) Obviously, the vertical distance between the points I and II of loop A as compared with point I of loop A and point III of loop B now indicates that the difference between two different slopes at the same outflow pressure can be augmented further, in this case from 8 to 10 mm Hg, by additional filling of the interstitial tissue spaces with capillary filtrate The displacement of the isovolumetric state to higher venous pressures (*i e* the intersections with the abscissa) is of course a reflection of the same phenomenon

The mean intestinal lymph flow measured in 3 cats was 0 55 ml/(30 min × 100 g intestine), increasing to 1 10 ml/30 min during an increase in venous outflow pressure of 20 cm water The corresponding mean outward filtration was 7 5 ml/(30 min × 100 g) It is important to stress that allowing for the drainage of lymph did not change the configuration of the volume recordings as seen in Fig 1

Discussion

The experiments show that the intestine has a great capacity to neutralize considerable changes in capillary pressure *E g* in Fig 1 a new isovolumetric state was reached 6—7 min after the venous outflow pressure was raised by 20 cm water Johnson and Hanson (1963) came to a similar result with their isogravimetric technique They plotted the weight changes after elevating the venous pressure on a semilogarithmic scale and found two distinct phases, a rapid one which they interpreted as corresponding to the venous distension and a slow one corresponding to the capillary filtration The rapid phase had a time constant of 10 9 sec, which means that after 3 time constants, ca 33 sec, 95 per cent of the venous filling was completed and after this time the volume increase consisted mainly of filtration This agrees very well with the present results where the same phenomenon is seen during the first 40—50 sec on the istope record of the blood volume Johnson and Hanson

did not discuss in their paper why the filtration also decreased exponentially, but in a later paper (Johnson 1965) it was explained as due to dilution of the interstitial proteins (see below). As, however, several different factors may contribute to this new Starling equilibrium at a higher capillary pressure level, such factors will now be discussed more at length.

The venous arteriolar response. When the portal venous pressure was elevated in dogs, the blood flow decreased relatively more than the perfusion pressure. Johnson (1959, 1960) showed that this was caused by a myogenic response, predominantly of the precapillary resistance vessels, which constricted when they were stretched by the raised pressure. This by now well known *response of many different types of* smooth muscle to stretch was originally suggested for vessels by Bayliss (1902). Two different effects of this venous arteriolar response are possible. 1. The precapillary constriction is effective enough to again lower the raised capillary pressure, caused by the primary rise in venous pressure (Johnson 1965). 2. The precapillary sphincters, which are known to be especially sensitive for pressure changes (Mellander, Öberg and Odelram 1964), constrict and therefore reduce the perfused capillary surface area, as determined by CFC. Such a reaction will reduce the extent of outward filtration even in case the capillary pressure rise would remain the same. However, it will not abolish the outward filtration except for the case that all capillaries become closed and the direction of the net fluid transfer can never be reversed by any changes in CFC. In general, it seems unlikely that a myogenic response to a sudden stretch, or unloading, would have a time course so prolonged as to give the smooth changes in filtration rate seen in Fig. 1 and 2. However, even if these myogenic responses are very important *per se* for counteracting primary changes in capillary pressure, they can not be significantly involved in these experiments for following reasons.

Isopropyl noradrenaline was always infused i.a. in such concentrations as to depress markedly, or even eliminate entirely the reactivity of the resistance vessels and venous arteriolar respones were therefore not seen in the present experiments. Also papaverine HCl in doses which are considered to abolish all vascular reactivity (Mellander and Lundgren 1966) did not decrease the maximal difference in net fluid transfer at the same outflow pressure. Moreover, in case there was some reactivity left after administration of these drugs it would then be expected that these 'active' adjustments would be of largely the same magnitude at equal rises of capillary pressure, whether these rises occurred during the phase of filtration or the phase of absorption of the loop. It follows that such active adjustments can not explain the considerable difference in direction of the slopes.

Plasma colloid osmotic pressure. A 3 kg cat has a plasma volume of about 150 ml (Hamlin and Gregersen 1939. The intestinal section used during the experiments weighed about 40 g and accumulated a maximum of 2—3 ml filtrate during the phase of outward filtration when a run of stepwise increased venous pressure was performed. Even in case the filtered fluid was totally protein free the plasma proteins would become only slightly concentrated by this fluid loss, corresponding to

an increase in the plasma colloid osmotic pressure by less than 0 5 mm Hg, a figure without significance for the present problem Furthermore, any increase in concentration of the plasma colloids will cause an inward absorption in other vascular beds, which will dilute the proteins again, thereby opposing the tendency to change the plasma colloid osmotic pressure

Tissue colloid osmotic pressure Dilution of the interstitial proteins lowers the interstitial colloid osmotic pressure and counteracts an elevated mean hydrostatic pressure in the capillaries Johnson (1965) reported that the dog intestine became isogravimetric at any venous outflow pressure between 0—18 mm Hg, and suggested that this was essentially due only to the dilution of the interstitial proteins, since tissue pressure, measured by a needle technique, was not affected even by large changes in venous pressure This will be commented on below

The present experiments have shown that differences in net fluid transport at the same venous outflow pressure correspond to a maximal change of often more than 10 mm Hg (maximally 14 8 mm Hg) in some factor, or factors, of the Starling equilibrium However, the effect of lowering the tissue colloid osmotic pressure must be limited by the initial value before the dilution This value must be very variable during different functional situations in the intestine, but a mean value of about 5 mm Hg was considered reasonable by Landis and Pappenheimer (1963) Even if the tissue colloid osmotic pressure in the intestine was considerably higher, theoretical difficulties remain Assume for a moment an interstitial colloid osmotic pressure as high as 15 mm Hg, which corresponds to a concentration of about 5 g of protein/100 ml To lower this pressure to 5 mm Hg the proteins must be diluted to 2 g/100 ml (Landis and Pappenheimer 1963) a dilution of the interstitial fluid approximately 2 5 times In the present experiments the difference in filtered fluid on that level of outflow pressure, which had the biggest difference in net fluid transfer, was very seldom more than 1—2 5 ml per 40 g intestine If a decrease of interstitial colloid osmotic pressure was the only factor involved, it would mean that only 0 67—1 67 ml per 40 g intestine (= 1 7—4 2 per cent) is interstitial fluid an improbably low figure

Moreover, it is very difficult to assume a *mean* value of proteins in the intestinal fluid as high as 4—5 g/100 ml Morris (1956) collected intestinal lymph from cats and found a protein concentration of 4 19 ± 0 29 g As in other tissues the concentration of proteins in the lymph ought to be considerably higher than that of the interstitial fluid Landis and Pappenheimer (1963) calculated that the continuously produced capillary filtrate in a resting animal was at an average at least 5—10 times greater than the average lymph flow For many reasons therefore, it seems impossible that dilution of the interstitial proteins could be responsible for more than 4—6 mm Hg of the deduced maximal difference in the Starling equilibrium This large difference up to about 14—15 mm Hg was, as mentioned, deduced from loops, as illustrated in *e g* Fig 4, where both the amount of filtered fluid and CFC are known

The tissue pressure As mentioned, Johnson (1964) did not find any increase in tissue pressure with the needle technique By a similar method Meyer, Nordheim and Ballin

(1962) found that the ...
did not increase du ... mm Hg, but increased signific ... during the edema of endotoxin shock. However, the methods to measure tissue pressure by needle techniques have been criticized for many reasons perhaps the most important one is the fact that even the finest needle is several hundred times larger than the tissue spaces, where the pressure should be recorded (McMaster 1946, Guyton 1963) When the connective tissue is disrupted by heavy edema then the spaces are probably more adapted to the recording needles On the other hand it is stated repeatedly that in many tissues outward filtration is counteracted by an increasing tissue pressure (Landis and Gibbon 1933, human forearm, McMaster 1946, mouse skin, Kjellmer 1965, cat skeletal muscle Theoretically, an increasing tissue pressure seems to be well adapted to the purpose of protection against edema The smaller the ... the inter...
when the ... fluid quantities other corrections will appe... (McMaster 1941, 1946) Perhaps such a disruption of tissue spaces can explain why, in infectious intestines, increased venous pressure causes outward filtration during very long time Tissue pressure can reach high values McMaster (1946, mouse skin) and Landis and Gibbon (1933, human forearm) found tissue pressures up to about 25 mm Hg with prolonged filtration When elevation of venous outflow pressure with 20—30 cm H_2O can be counteracted so that the outward filtration ceases, it seems very unlikely that this can be explained without considerable contribution of a raised tissue pressure

In the present investigation the lymphatics were ligated in most cases (as in Johnson's experiments) and it is technically difficult to record lymph flow and intestinal volume simultaneously It may then be questioned whether the assumed rise in tissue pressure is simply an artifact caused by the accumulation of lymph fluid It is a well known fact that raised venous pressure will increase the rate of lymph production However, there is usually a considerable delay before the rate of lymph flow increases to an extent that equals the rate of outward filtration Morris 1956) found an intestinal lymph flow in resting cat of about 4 ml per hr After raising the portal pressure with 12 cm water it increased to 6 ml per hr but needed 60 min to reach this value During the first 15 min period it amounted only to about 4.5 ml per hr, i e an increase with 0.5 per hr for a rise of 12 cm water = 0.008 ml per min Assuming 100 g intestine and a mean CFC of 0.15 a rise of 12 cm water in outflow pressure, corresponding to 7.5 mm Hg increase in mean capillary pressure should cause an outward filtration amounting to 0.15 · 7.5 mm Hg = 1.13 ml per min if no counteracting factors become secondarily involved

Since Morris' data were very extensive lymph was collected in only 3 experiments in the present investigation where simultaneously tissue volume was recorded The mean rise in lymph flow was 0.55 ml per 30 min for 20 cm elevation in venous pressure, a figure quite comparable with those given by Morris There is so grea discrepancy between the calculated outward filtration in case no counter...

are involved, and the corresponding measured increase in lymph flow, that ligating the lymph vessels can not be an important cause for the increase in tissue pressure deduced within periods of 10—15 min, when venous outflow pressure was raised

The present investigation has shown that the intestine is fairly effectively protected against edema during increases in venous outflow pressure, in spite of a very big capacity for net fluid transfer across the capillary wall Differences in the Starling equilibrium up to about 14—15 mm Hg was deduced by the method of stepwise raising and lowering the venous pressure It seems impossible to explain th s differ ence without assuming an increasing tissue pressure when fluid is filtered into the tissue spaces In the living animal, however, more mechanisms than tissue pressure and tissue colloid osmotic pressure, e g the venous arteriolar response, certainly play an important role in decreasing the potential risk of having the big capillary transfer capacity necessary as it is for the primary functions of the small intestine

This study was supported by grants from Air Force School of Aerospace Medicine under Contract AF 61(052) 732 through the European Office of Aerospace Research (OAR) United States Air Force from U S Public Health Service (HE 05675 05 06) and from the Faculty of Medicine, University of Goteborg

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Acta physiol scand. 1966. 68 316—321
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Effect of Finnish Bath (Sauna) on the Urinary Excretion of Noradrenaline, Adrenaline and 3-Methoxy-4-Hydroxy-Mandelic Acid

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Received 28 April 1966

Abstract

Huikko, M , P Jouppila and N T Kärki *Effect of Finnish bath (Sauna) on the urinary excretion of noradrenaline, adrenaline and 3 methoxy 4-hydroxy-mandelic acid* Acta physiol scand 1966 68 316—321

For the present work the urinary excretion of noradrenaline adrenaline and 3 methoxy 4 hydroxy

Many authors have shown that urinary excretion of noradrenaline increases as a result of physical effort (Euler and Hellner 1952, Kärki 1956), but also in situations of emotional tension anger or aggressions without physical exertion (Elmadjian *et al* 1958, Levi 1961) Adrenaline excretion may also rise in these situations, though it is specifically increased by anxiety unpleasant emotions and stress from work (Euler *et al* 1959, Pekkarinen *et al* 1961 Levi 1961)

On the other hand a pleasing situation such as watching a scenic film has been found to reduce significantly the excretion of adrenaline and noradrenaline (Levi 1963)

The Finnish sauna offers a special field for the study of catecholamine metabolism Almost every Finn takes a sauna once a week The majority find it emotionally a pleasant, relaxing experience, but it produces major changes in the circulatory system The period spent in the sauna usually ranges from 30 to 60 min According to Eisalo (1956), body temperature, recorded rectally, increases by 1—1 5° C due to the heat of the surroundings Cutaneous blood vessels are dilated, and peripheral resistance falls significantly Cardiac output in healthy subjects increases by 73 per

TABLE I Excretion of noradrenaline adrenaline and 3 methoxy-4 hydroxy mandelic acid during a Finnish bath (sauna)

Group	Subject	Noradrenaline excretion ng/min		Adrenaline excretion ng/min		3 methoxy 4 hydroxymandelic acid excretion µg/min		Pulse beats per min	
		Control	Sauna	Control	Sauna	Control	Sauna	Control	Sauna
I	1	29.6	66.5	7.1	9.5	6.0	7.4	82	124
(Controls	2	9.1	71.7	3.1	12.1	3.9	3.4	52	124
after	3	13.3	81.9	6.2	14.1	4.2	3.9	75	122
sauna)	4	8.1	35.8	13.0	6.9	3.0	4.3	76	120
	5	24.6	66.5	6.1	6.1	6.3	6.0	90	130
	6	36.1	47.8	11.3	11.7	3.7	4.4	72	120
	7	32.0	42.0	3.0	5.0	4.4	4.8	—	—
	8	66.0	54.0	6.0	10.5	4.7	3.6	—	—
	9	51.0	51.3	3.7	5.7	6.2	4.2	62	86
II	10	40.3	33.0	8.4	21.0	3.8	5.3	80	83
(Controls	11	40.6	26.9	6.6	17.1	3.6	4.4	72	134
24 hrs	12	23.4	48.0	7.6	13.0	3.2	6.9	52	140
later)	13	36.0	25.8	4.8	5.8	—	—	74	115
	14	39.0	39.6	9.5	6.9	—	—	62	82
	15	35.1	58.2	11.6	11.8	—	—	96	140
	16	18.0	28.2	5.4	3.8	—	—	70	96
	17	29.2	38.5	6.8	5.5	1.7	3.2	—	—
	18	19.5	23.1	5.5	6.4	3.8	3.2	—	—
Mean and S E M		30.6± 3.4	46.6± 4.1	7.0± 0.7	9.6± 1.1	4.2± 0.3	4.6± 0.4	72.5± 3.4	115± 5.3

cent, and pulse rate by average of 61 per cent Systolic and diastolic blood pressure remain practically unchanged

In the present work the changes in the urinary excretion of noradrenaline, adrenaline and their major metabolite, 3 methoxy-4 hydroxy mandelic acid due to the sauna bath has been studied

Material and Methods

The experiment comprised 18 healthy male subjects mean age 24 years all of whom habitually took the sauna where for this study they stayed 25—40 min at a temperature of 70—80 C and relative humidity of 20—40 per cent

Before starting the bath the resting pulse rate blood pressure and sublingual temperature of 14 test subjects was recorded These recordings were repeated in the sauna after 20 min immediately on leaving the sauna and 20 min later

Norad [illegible] test subjects emptied their bladders [illegible] Urine passed during the 2—3 hrs [illegible] re collected during the 2—3 hrs [illegible] other 10 subjects during a [illegible] corresponding to that of the sauna 24 hrs later

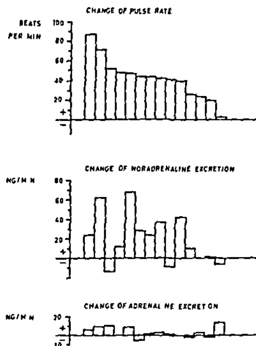


Fig 1 Changes in the pulse rate noradrenaline and adrenaline excretion in 14 test subjects during a Finnish bath (Sauna)

The pH of the collected urine sample was adjusted with 6 N hydrochloric acid to about 3 and the specimens were kept in the refrigerator until the determinations were performed At this pH and temperature the catecholamine content remains constant over long periods (Karki 1956, Euler and Lishajko 1961)

The method for differential estimation of noradrenaline and adrenaline described by Crout (1961) was used This method consists of adsorption of catecholamines in alumina at pH 8 4, elution with 0 2 N acetic acid oxidation with iodine at pH 6 5 and 3 5 into aminochromes and

3 methoxy 4 hydroxy mandelic acid was determined according to Pisano *et al* (1962) by extracting the metabolite from the urine oxidating it with periodate into vanillin and measuring the vanillin content with spectrophotometer at 360 mμ A standard of 10 μg 3 methoxy 4 hydroxy mandelic acid was carried through the entire procedure in every series

Creatinine was determined from the urine of 6 subjects using the alkaline picrate method (Reiner 1953)

Results

A *Excretion of noradrenaline, adrenaline and 3-methoxy 4 hydroxy mandelic acid into the urine*

The mean value of noradrenaline excretion for control group I (after the sauna) was 27 3 ± 7 2 ng/min and for control group II (at the same hour 24 hrs later) 33 2 ± 3 ng/min The difference between the readings for control groups I and II was not significant and therefore the mean of all control readings was used Average excretion of noradrenaline into urine increased during the sauna, from the mean control value of 30 6 ± 3 4 ng/min to 46 6 ± 4 1 ng/min (Table I) This change is statistically significant ($P < 0\ 02$) Adrenaline excretion rose from 7 0 ± 0 7 ng/min to

9 6 ng/min, the difference is significant ($P < 0.05$) The control value obtained for 3 methoxy-4-hydroxy mandelic acid was 4 2 ± 0 3 ug/min and the sauna value 4 6 ± 0 4 ng/min The difference here is not significant ($P < 0.3$)

B *Change in pulse rate*

The resting pulse rates of the subjects ranged from 52 to 96 beats per minute, the average was 72 beats per minute During the sauna, all test subjects showed an increase in pulse rate, by an average of 43 beats per minute ($P < 0.001$)

The change in the excretion of catecholamines in the 14 different test subjects is shown in Fig 1 in the order in which the pulse rate increased

C *Blood pressure*

Changes in the blood pressure during the sauna were slight Control value recorded in sitting position ranged from 110/75 to 155/100 mm Hg, and the mean value was 130 85 mm Hg The systolic pressure rose in the sauna by an average of 10 mm Hg, while the diastolic remained unchanged

D *Body temperature*

Body temperature, recorded sublingually, increased in the test subjects by 0 1—0 3° C

E *Creatinine excretion and catecholamines*

The creatinine values of test subjects 1—6 averaged 55 8 mg per hr during the control period and 72 0 mg per hr in the sauna, there was thus a distinct increase The increases in the catecholamine excretion per time unit and per creatinine excretion were approximately of the same order the total catecholamine excretion per time unit increased as a result of sauna by 155 per cent and per mg of creatinine by 140 per cent

Discussion

A comparison of the changes noted in circulation in this study, with Eisalo s 1956) findings, shows that he recorded an identical rise in pulse rate He also showed that the systolic and diastolic blood pressure of a healthy subject remained, on the average unchanged in the sauna, while the present authors recorded a slight rise in systolic blood pressure The recorded control values for catecholamine excretion correspond in magnitude to those mentioned by other authors It was also found that the control values taken immediately after the sauna sample and those obtained on the following day at the same hour were not significantly different This agrees with the findings of Karki (1956) who stated that the excretion in the same person remains nearly constant at the same hours on different days

Bearing in mind the circulatory changes which are shown to be produced body heating, and

present results which show that the sympathetic tone is involved in the sauna seem logical As a result of surrounding heat the temperature of the skin increases, and due to reflex release of vasoconstrictor tone the cutaneous blood flow increases (Roddie *et al* 1956) Apparently the widening of the vascular bed in the skin is compensated for by vasoconstriction in other areas to maintain blood pressure homeostasis The observation that blood flow through internal organs diminishes during body heating supports this view (Grayson 1951) The circulatory changes are transmitted by sympathetic nerves, this higher level of activity leads to an increased liberation of noradrenaline from nerve endings and an increased excretion in urine

Although factors known to increase adrenaline release are slight or missing, the slight increase in adrenaline excretion indicates that also adrenal medulla is stimulated The percentage of adrenaline from total catecholamines was practically the same in sauna samples as in controls

The excretion of 3 methoxy-4 hydroxy mandelic acid has been found to reflect short term sympathetic activity less well than does the excretion of free noradrenaline and adrenaline (Euler 1964) This was also revealed by the present study in that the increase in the excretion of 3 methoxy 4 hydroxy mandelic acid was distinctly smaller and was not statistically significant This phenomenon is attributed to the relative slowness of 3 methoxy 4 hydroxy mandelic acid excretion (Euler 1964)

Little is known of the part played by the kidneys in the excretion of catecholamines and their metabolites but more of the influence of the sauna on renal function Karvonen (1954, 1955) has attributed reduced urine excretion in the sauna to an increased liberation of antidiuretic hormone A sauna bath of normal duration reduces glomerular filtration slightly but plasma flow is affected more deeply (Haapanen 1958) Those changes are considered to be due to the constriction of efferent glomerular arteries and are similar to the changes produced by injection of vasopressor substances Whether or not those slight changes in renal function in the sauna affect the excretion of catecholamines remains to be elucidated

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The Effect of DOPA on the Spinal Cord

3. Depolarization Evoked in the Central Terminals of Ipsilateral Ia Afferents by Volleys in the Flexor Reflex Afferents

By

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Received 4 May 1966

Abstract

The effect of DOPA on the spinal cord 3 Depolarization evoked in the central terminals of ipsilateral Ia afferents by volleys in the flexor reflex afferents Acta physiol scand 1966 *68* 322—336

In unanaesthetized spinal cats injected with l DOPA volleys in the flexor reflex afferents (FRA) evoke a long latency long lasting dorsal root potential (DRP) Measurements of excitability of ipsilateral primary afferent terminals show a corresponding depolarization of Ia but not Ib or cutaneous afferent terminals Volleys in the FRA have no effect on Ia afferent terminals in the normal acute spinal cat presumably because the neuronal pathway transmitting the effect from the [illegible]

DOPA and is probably caused by a removal of depolarization

In the acute spinal cat Ia afferent terminals are depolarized by volleys in Ia and Ib primary afferents, especially from flexor muscles (Eccles, Magni and Willis 1962), but not by stimulation of the flexor reflex afferents (FRA) Volleys in the FRA normally produce some depolarization of Ib terminals (*Eccles, Schmidt and Willis* 1963a) but their main effect is that exerted on their own terminals here presynaptic inhibition acts as a negative feedback (Eccles, Kostyuk and Schmidt 1962 Eccles, *Schmidt and Willis 1963b, Lund, Lundberg and* Vyklický *1965)*

The depolarization of primary afferent terminals is profoundly changed after intravenous injections of *l*-3,4 dihydroxyphenylalanine (DOPA) The normal dorsal root potentials (DRP) evoked by volleys in the FRA are depressed but an additional DRP appears which has a longer latency and a much longer duration (Andén *et al*

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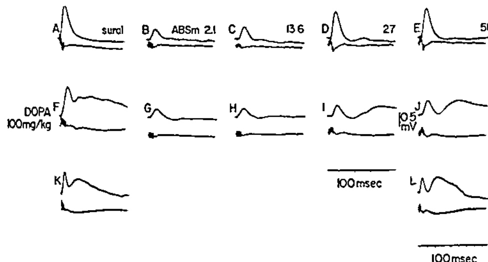


Fig 1 The effect of DOPA on the DRPs evoked from the FRA The upper traces show the DRPs recorded from the most caudal dorsal rootlet in L6 the lower traces are recorded from the L7 dorsal roo

...pp ...s to all DRPs

1966a) In the present paper it will be shown that the late DRP is caused by depolarization of the terminals of Ia afferents, therefore a previously undetected neuronal pathway exists in the spinal preparation, which permits depolarization of Ia terminals by volleys in the FRA Some of the results have been briefly reported (Anden *et al* 1964)

Methods

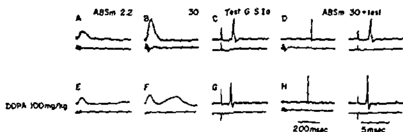
The experiments were made on unanaesthetized spinal cats as described by Anden *et al* (1966a) Excitability measurements from primary afferent terminals were made as described by Wall (1958)

The following abbreviations are used dorsal root potential DRP primary afferent depolarization PAD posterior biceps semitendinosus PBSt anterior biceps semimembranosus ABSm plantaris Pl, gastrocnemius soleus G S posterior knee joint nerve J sural Sur flexor reflex afferents FRA

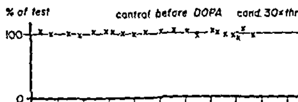
Results

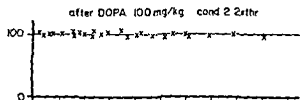
1 *The DRPs evoked from the FRA after DOPA*

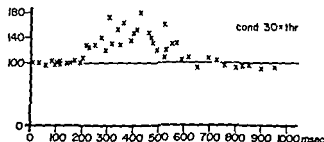
Trains of volleys were employed to evoke the DRPs shown in Fig 1 The control records A-E, before DOPA was given, show the normal early DRPs with a duration of about 200 msec DOPA reduced the early DRPs from cutaneous afferents (F) and from high threshold muscle afferents (I and L but a late DRP appeared whose latency is about 100 msec in I and 150 msec in J Records K and L taken at slower speed show the long dur



ABSm → G-S Ia







G and the right hand records of D and H under

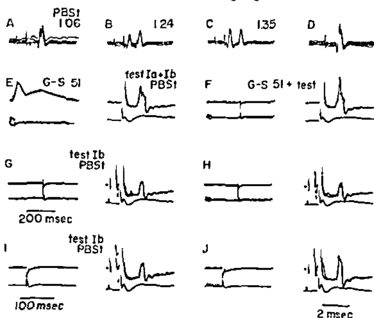
low threshold cutaneous afferents did not evoke the late DRP, it appeared only when the stimulus strength was raised to about twice threshold. Volleys in group I muscle afferents never produced a late DRP and the muscle nerves usually had to be stimulated at a strength of 20—30 times threshold in order to evoke the late PAD. However, sometimes a late DRP could be evoked from group II afferents and occasionally from very low threshold group II afferents (Fig. 6).

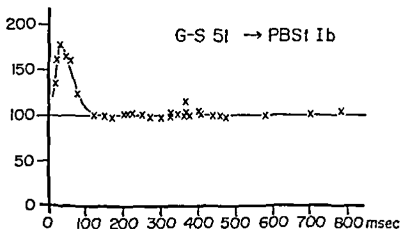
In a few of the experiments the late DRP failed to appear after DOPA had been given. It is of interest that in those experiments DOPA nevertheless effectively depressed the early DRP evoked from the FRA.

2. *The recipient primary afferents*

Excitability measurements were made to disclose which primary afferents were depolarized when the late DRP was evoked after DOPA had been injected. In the experiment of Fig. 2 the tip of the stimulating microelectrode was located in the G S motor nucleus and the antidromic Ia volleys recorded peripherally in the G S nerve. After DOPA a train of volleys in high threshold muscle afferents increased the excitability of the Ia terminals. The latency and time course of this increased excitability paralleled the late DRP evoked by the same train of volleys. There does not seem to be any difference in receptiveness between Ia terminals from extensor and flexor muscles, in the same experiment an equally large increase in excitability was found in the terminals of Ia afferents from the flexor PBSt (cf. Fig. 9).

Fig. 3 illustrates an experiment in which the excitability of primary afferent terminals was tested with the tip of the stimulating electrode in the region of the intermediate nucleus of the spinal cord, where the extracellular focal potential evoked from Ia and Ib afferents was maximal. The group I volley from PBSt had good separation in Ia and Ib components (A, D) and the antidromic spike evoked from the intraspinal stimulating electrode and recorded in the PBSt nerve had a double configuration (E). The early component of the antidromic spike was a Ia volley, as shown by its abolition (record G and I) when a colliding maximal Ia volley was evoked from a second pair of electrodes on the PBSt nerve. The remaining antidromic volley was in Ib afferents as was proved by its disappearance with a maximal group I colliding volley. A train of volleys in high threshold muscle afferents which produced a late DRP (E) did not change the size of the Ib antidromic test volley (H and curve). Hence there was no increase in the excitability of the Ib terminals corresponding in time to the late DRP. The effect in J at a short conditioning testing interval the time course of which can be seen in the early part of the curve, is the normal effect of group Ib muscle afferents (Eccles *et al.* 1963a) and shows that it was possible to obtain an increased excitability in Ib afferents in this experimental situation. Hence there is no evidence that a PAD in Ib afferents contributes to the late DRP after injection of DOPA. Fig. 3 F shows the increased excitabi[lity of]
Ia afferents when the test stimulation was applied in the region of the
nucleus. It is likely that the increased excitability in this case was caused


DOPA 100mg/kg
A
PBSt
1 06
B
1 24
C
1.35
D
E
G-S 51
test Ia+Ib
PBSt
F
G-S 51 + test
G
test Ib
PBSt
H
200 msec
I
test Ib
PBSt
J
100 msec
2 msec


% of test
G-S 51 → PBSt Ib
200
150
100
50
0
0 100 200 300 400 500 600 700 800 msec

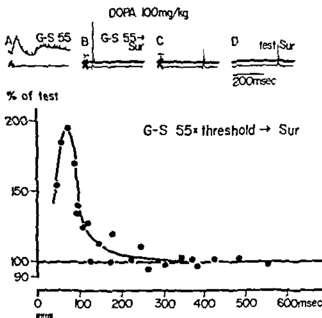


Fig 4 Measurement of the excitability of cutaneous afferent terminals when conditioned by volleys in the FRA after DOPA Record A shows that a long latency DRP (upper trace) was evoked from high threshold muscle afferents after injection of DOPA (100 mg/kg), the lower traces are recorded from the L7 dorsal root entry zone Records B D show the antidromic volley recorded from the sural nerve evoked by local stimulation within the dorsal horn where the extracellular monosynaptic focal potential obtained by stimulation of the sural nerve was maximal When conditioned by volleys in the high threshold afferents of the G S nerve the test antidromic volley is increased at short conditioning test intervals (B), but not at longer intervals (C) corresponding to the late wave in the DRP, the time course of the increased excitability is plotted as in Fig 2

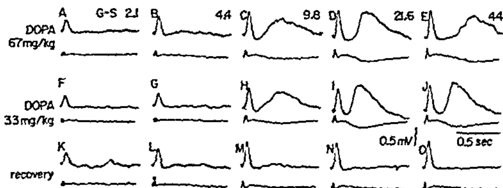
synaptic effects on the Ia terminals in this region The PAD in these more dorsally located Ia terminals may very well give a major part of the late DRP

The excitability of cutaneous afferent terminals was tested by stimulation through an electrode whose tip lay at the level in the dorsal horn where a large monosynaptic extracellular focal potential was evoked by stimulation of cutaneous afferents In Fig 4 there is an initial increase in excitability caused by the early PAD from Ib afferents and from the FRA (cf Eccles *et al* 1963b) but there was never any increased excitability in cutaneous afferents during the late DRP No attempt was made to find out whether high threshold muscle afferents are depolarized from the FRA during the late DRP However, this seems unlikely since cutaneous afferents and high threshold muscle afferents usually receive similar effects from the FRA Hence our findings suggest that the late DRP evoked from the FRA after injection of DOPA is caused exclusively by a PAD in Ia afferents

In some experiments stimulation of the FRA evoked a late wave in the DRP and a long latency discharge in the ventral roots without any DOPA being given in addition there was always a depression of the usual short latency effects from the FRA Measurements of the excitability of the terminals of Ia Ib and cutaneous afferents showed that, at a time corresponding to the late DRP the excitability of the Ia terminals was increased by volleys in the FRA but not in group I afferents No

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afferents of the G S nerve The time course of the increased excitation of Ib afferents is plotted Fig 2 Time calibration for the left hand records of E-H is shown in G and for the left hand in I and J in I All right hand records in E J were taken at the faster speed in J



(67 mg/kg) and demonstrate the appearance of the late DRP with activation of the FRA (C) and *the increase of its latency as stimulation strength was increased (C–E). This latter effect was not so* marked (H–J) after an additional 33 mg DOPA/kg had been injected. Records K–O were taken 2 hrs later when the effects of the DOPA injections had worn off and no late wave could be evoked by activation of the FRA. Time scale applies to all records, voltage calibration to all DRPs.

increased excitability of Ib or cutaneous afferent terminals at this latency was found. It seems that under some conditions modifications of spinal reflexes may occur in the normal acute spinal preparation which usually only occur after injection of DOPA.

3. *Inhibition of the late DRP*

Fig. 5 shows that the latency of the late DRP is prolonged when the strength of stimulation is increased. After injection of 67 mg/kg of DOPA the magnitude of the late DRP increased somewhat when the stimulus strength was raised from 9.8 to 21.6 times threshold (D). With further increase of the stimulus strength (E) the most marked effect was a prolongation of the latency but there was also some decrease of *the size of late DRP. F–J were taken after an additional dose of DOPA, 33 mg/kg.* The DRP evoked at 9.8 times threshold in H was almost the same as in C but with increase of stimulus strength in I and J the prolongation of the latency, although still present, was not so marked as in D and E. Furthermore there was no decrease of the late DRP in J when the stimulus strength was raised to 44 times threshold. Records K–O were taken 2 hrs later when the cat had recovered from the effect of DOPA. The early DRPs from the FRA had increased slightly and were not followed by late waves. The decrease in magnitude of the late wave with increasing strength of stimulation may be more marked than in Fig. 5. In the experiment of Fig. 6 the late DRP was evoked from low threshold group II afferents (B) and when group III afferents were also stimulated (D) the late wave was almost abolished. Correspondingly there was a removal of the increased excitability in the Ia afferent terminals with increased strength of stimulation (lower records). From these results it is inferred that stimulation of the FRA not only evokes the late DRPs but can also inhibit them. Another indication of this inhibition is given with prolonged repetitive stimulation

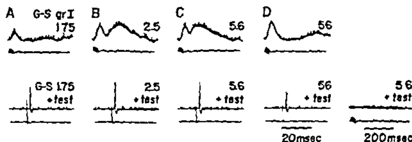


Fig 6 [illegible]

the DRP [illegible]

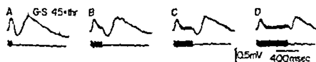


Fig 7 The effect of prolonging repetitive stimulation on the latency and amplitude of the late wave in the DRP evoked after DOPA Upper traces show the DRPs, lower traces the potentials recorded from the dorsal root entry zone, evoked by repetitive stimulation of the G-S nerve at 45 times threshold after injection of DOPA (67 mg/kg) Note both the increase in latency of the late wave of the DRP and the decrease in amplitude. Same time scale for all records, the voltage scale applies to all DRPs

of the FRA Fig 7 shows that whatever the duration of the repetitive stimulation the late wave is not evoked until after cessation of stimulation, and it also demonstrates that while a short train of volleys in the FRA may evoke a substantial late DRP, increasing the length of the train may decrease the amplitude of late wave

4 *Hyperpolarization of primary afferents after DOPA*

There is sometimes evidence that after injection of DOPA volleys in the FRA produce a positive DRP In Fig 8 DOPA as usual depressed the early DRP evoked from the FRA and produced a late DRP However, interposed between these two components of the DRP there was also a positive potential which was clearly evoked from the FRA since there was hardly any positivity from group I afferents (D) but an increasing effect (in E-G) when the stimulus strength was increased Record C sh that the positive DRP can also be evoked by a single volley in high threshold afferents, but in this case it was not followed by a late DRP

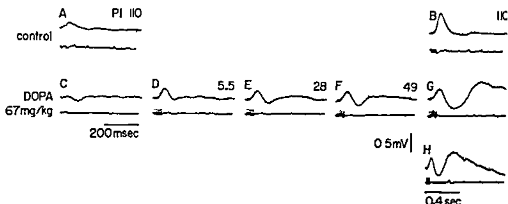


Fig 8 Positivity in the DRP evoked from the FRA after DOPA The upper traces show the DRPs the lower traces are recorded from the dorsal root entry zone The PI nerve was stimulated with

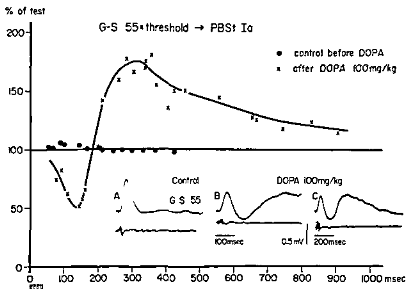
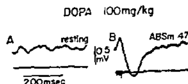


Fig 9 The correlation after DOPA between the positive and late negative waves in the DRP and the excitability of Ia afferent terminals Upper traces of records A C show the DRP evoked by stimulation of the G-S nerve to activate high threshold afferents before (A) and after (B C) injection of DOPA (100 mg/kg) the lower traces are recorded from the dorsal root entry zone The graph ... n the ... ngth ... that ... and late negative waves in the DRP (cf curve and C) Time scale the same for A and B voltage calibration applies to all DRPs



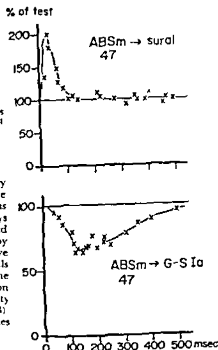


Fig 10 Measurements of the excitability of cutaneous and Ia afferent ... lower traces are recorded from the dorsal root entry zone The upper curve shows the time course of the increase in excitability of the terminals of cutaneous afferents of the sural nerve when conditioned by volleys in the high threshold afferents of the ABSm nerve plotted as in Fig 2 The test antidromic volley was evoked by local stimulation within the dorsal horn The lower curve shows the excitability changes of Ia afferent terminals of the G S nerve with the same conditioning volleys The test antidromic volley was evoked by local stimulation within the G-S motor nucleus The decreased excitability corresponds well in time to the positivity in the DRP (B) Same time scale for A and B, voltage calibration applies to the DRPs

Excitability measurements have shown that the positive DRP is associated with a decreased excitability in Ia afferent terminals In Fig 9 a train of volleys in high threshold muscle afferents gives an initial decrease in the Ia excitability followed by the later long lasting increase On the other hand, no decrease in excitability was observed in Ib or cutaneous afferents during the positive DRP

Fig 10 is from an experiment in which the positive DRP evoked from high threshold muscle afferents was exceptionally large and not followed by a late nega tive wave The excitability measurements in the lower curve show a long lasting decreased excitability in Ia afferents whereas in cutaneous afferents upper curve there is only the early increased excitability, with no evidence of a later decrease in excitability Since there was no late negative wave in this experiment it is assumed that record B and the lower curve give the time course of the primary afferent hyperpolarization when not interrupted by the late PAD Fig 11 is from another experiment in which again there was a predominantly positive DRP evoked from the FRA after DOPA had been injected A and B) The group I volley from PBSt had separation into Ia and Ib components (C), and the antidromic volley ev

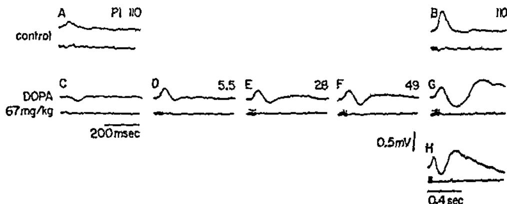
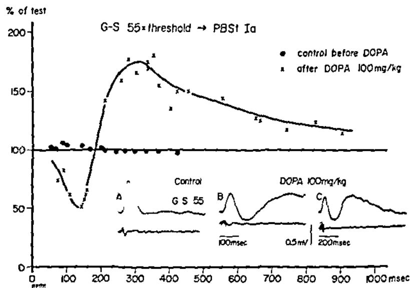


Fig 8 Positivity in the DRP evoked from the FRA after DOPA The upper traces show the DRPs the lower traces are recorded from the dorsal root entry zone The P1 nerve was stimulated with

both the positive wave and the late negative wave is shown in D G, the early negative wave is unaltered and evoked only by group I afferents Record A shows the small early negative DRP evoked by a single volley in the FRA which is suppressed by injection of DOPA, a single volley in the FRA now gives only a small positivity (C) Same time scale for records A G Voltage scale applies to all DRPs



DRP and

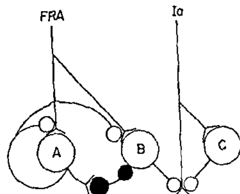


Fig 12 A schematic representation of the neuronal pathways involved in the depolarization of Ia afferent terminals evoked by stimulation of the FRA Pathway A is activated in the acute spinal cat and inhibits pathway B so that normally no depolarization of Ia terminals results from activation of the FRA The black interneurone is inhibitory but it is not known if it operates postsynaptically or presynaptically by depolarizing the terminals of interneurones Pathway A can be partially or completely suppressed by injection

for the depolarization of Ia terminals evoked by activation of Ia afferents, it may share interneurones with pathway B (see Discussion) A single interneurone in the diagram may represent a chain of interneurones

terminals (Eccles 1964) This selective action is completely reversed during the late long lasting DRP evoked from the FRA after i v injection of DOPA only Ia afferent terminals (from flexors and extensors) are depolarized and there is no effect on Ib or on cutaneous afferent terminals To judge from the size of the DRP and the excitability increase this PAD from the FRA may be as large as that evoked by volleys in group I afferents from flexors (Eccles, Magni and Willis 1962) The latter action gives presynaptic inhibition of transmission from Ia afferents (Frank and Fuortes 1957, Eccles, Eccles and Magni 1961) and it is assumed that the PAD evoked from the FRA also has an inhibitory effect on transmission from Ia afferents The functional significance of this inhibitory action in motor regulation will be considered in a forthcoming paper on the late reflex activation of motoneurones, which can be evoked after injections of DOPA (cf Jankowska *et al* 1965, Lundberg 1965, 1966) The main object of the following dicussion is to consider why volleys in the FRA have no effect on Ia afferents in the normal acute spinal preparation and how it comes about that there is an effect after DOPA has been given It is postulated that this problem is essentially one of inhibitory interaction between spinal reflex pathways from the FRA

The neuronal linkage between the FRA and primary afferent terminals is shown schematically in Fig 12 Without DOPA volleys in the FRA give a PAD in their own terminals (Eccles, Kostyuk and Schmidt 1962 through the interneuronal pathway marked A B indicates the interneuronal pathway from the FRA to Ia afferents disclosed by DOPA and which is responsible for the long latency PAD This scheme makes no attempt to account for the fact that excitatory and inhibitory effects of very different latencies and durations may be produced this problem will be discussed in a later paper Our results indicate that there is no transmission in pathway B when A is active and *vice versa* no transmission in pathway A when B is active This interrelationship suggests an inhibitory interaction between pathway A and B One possibility would be that DOPA facilitates pathway B (which will

not function without a facilitatory support) and that when this pathway is active it inhibits pathway A. However, the alternative linkage of an inhibition from A to B is shown in Fig. 12, this hypothesis is preferable for the following reasons: 1) DOPA may occasionally depress the short latency path A without opening pathway B, hence the depression of A does not seem to depend on activity in B. 2) On increasing the stimulus strength it is sometimes observed that the effect via pathway B is decreased (Fig. 5 and 6), 3) During prolonged repetitive stimulation of the FRA transmission in B is depressed (Fig. 7). All these observations would be expected with the direction of inhibitory interaction indicated in the diagram of Fig. 10 but are difficult to explain by an inhibition from B to A.

It has been suggested that DOPA modifies the effects of volleys in the FRA by liberating transmitter from a descending noradrenergic pathway, which inhibits transmission in the short latency paths from the FRA (Andén *et al.* 1966 a, b). Engberg and Ryall (1965, 1966) have postulated that noradrenaline is an inhibitory transmitter in the spinal cord and that the descending noradrenergic pathway inhibits transmission in the short latency FRA pathway by a monosynaptic action. It is now suggested that DOPA acting through the descending noradrenergic system inhibits transmission in pathway A and in doing this removes the inhibitory effect on pathway B thereby permitting transmission via this pathway from the FRA to Ia afferent terminals. It is necessary to emphasize the tentative nature of the hypothesis that DOPA acts via a descending noradrenergic pathway (cf. Andén *et al.* 1966 b), further investigations are required to establish the exact mechanism of its action, but a detailed knowledge of the mechanism by which DOPA acts is not required for the discussion of the main problem which is the interaction between paths A and B in Fig. 12. The important factor in releasing path B seems not to be that DOPA is given but that transmission in the short latency path A is depressed. This is indicated by our finding that a late PAD in Ia afferents was evoked from the FRA in two spinal cats to which DOPA had not been given but in which transmission in the short latency pathways from the FRA was depressed for unknown reasons. It should, however, not be assumed that any depression of the short latency path from the FRA will necessarily give a release of the pathway from the FRA to Ia afferents. For example there is usually no release of transmission in the latter pathway during the tonic decerebrate inhibition of transmission in the short latency paths from the FRA (Eccles and Lundberg 1959, Carpenter *et al.* 1963). The explanation may be either that in this state there is descending inhibition both of pathway A and B or else that the first interneuronal relay (not shown in Fig. 12) is common for both these pathways and that the decerebrate inhibition is exerted at this level. If so, the release of transmission from the FRA to Ia afferents should only occur when the second order interneurones in the short latency path from the FRA are inhibited. The reason why it is important to consider different schemes for the interneuronal linkages from the FRA is the accumulating evidence that there are several descending pathways controlling transmission from the FRA (Lundberg 1966). Because of the interaction between the various interneuronal reflex pathways the significance of a

descending facilitatory or inhibitory system may depend entire[illegible] where in the interneuronal chain the effect is exerted

According to Fig 12 interneurones in path B receive both excitation and inhibition from the FRA In recent experiments intracellular recording from interneurones has demonstrated that many interneurones receive *postsynaptic* inhibition from the FRA, convergence of excitation and inhibition from the FRA was observed in several interneurones (Hongo, Jankowska and Lundberg 1965, 1966) but there may probably also be *presynaptic* inhibition through depolarization of synaptic terminals from interneurones (cf Lund *et al* 1965) Inhibition of pathway B is also of interest in relation to the long latency of transmission from the FRA to Ia afferents Transmission in pathway A is presumably only partially inhibited by DOPA and a synchronized volley in the FRA may delay transmission through pathway B by means of the inhibition exerted from A in Fig 12 The observation that an increased strength of stimulation may prolong the latency of the PAD in Ia afferents supports this interpretation This interpretation implies that the inhibition of the late DRP Fig 5 and 6) is an indication of the inhibitory mechanism that normally prevents the production of the late PAD in Ia afferents

The inhibitory effects are probably also significant in relation to the selective hyperpolarizing action in Ia afferent terminals that volleys in the FRA may have after DOPA (Fig 8—11) If for some reason there is a continuous activity in pathway B the Ia afferent terminals may be kept in a depolarized state Under such conditions the inhibitory effect from the FRA via pathway A may stop the activity in pathway B and thereby repolarize the Ia afferent terminals Similar mechanisms have been proposed as explanations for the primary afferent hyperpolarizations evoked from C fibres (Mendell and Wall 1964) and from the brain stem (Lundberg and Vyklicky 1963, 1966)

One problem remains to be discussed in relation to inhibition of transmission to Ia afferents Fig 12 C shows the pathway by which Ia afferents may produce PAD in Ia terminals Transmission in this pathway can be very effectively inhibited from the FRA (Lund *et al* 1965) this inhibitory effect is not indicated in Fig 12, where B and C are shown as separate pathways, but they may have interneurones in common and, if so, it should be considered if the inhibitory effect of Lund *et al* (1965) on pathway C may be identical with that exerted on pathway B in Fig 10 In this connection it is of interest that also the inhibitory effect of Lund *et al* (1965) is depressed after DOPA

This work was supported by the Swedish Medical Research Council (Project No 14X 94-01 and 02 N)

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Acta physiol. scand. 1966. 68. 337—341

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The Effect of DOPA on the Spinal Cord

4 Depolarization Evoked in the Central Terminals of Contralateral Ia Afferent Terminals by Volleys in the Flexor Reflex Afferents

By

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Received 4 May 1966

Abstract

JANKOWSKA, E, S LUND and A LUNDBERG The effect of DOPA on the spinal cord 4 *Depolarization evoked in the central terminals of contralateral Ia afferent terminals by volleys in the flexor reflex afferents* Acta physiol scand 1966 *68* 337—341

After injection of DOPA in unanaesthetized spinal cats volleys in the flexor reflex afferents (FRA) evoke ... of contra later ... cutaneous affer ... to contra later

In previous papers in this series it has been shown that after an i v injection of 3,4 dihydroxyphenylalanine (DOPA) volleys in the flexor reflex afferents evoke a late ipsilateral dorsal root potential (DRP) which is caused by a selective depolarization in the central terminals of Ia afferents (Anden *et al* 1966a b) In the present paper it will be shown that after DOPA volleys in the FRA produce similar effects on the contralateral side The experimental procedures have been given in the previous papers

Results

The DRPs in Fig 1 are evoked by short trains of stimuli After an intravenous injection of DOPA the short latency DRPs evoked from contralateral cutaneous afferents (F) and high threshold muscle afferents I—J) are depressed (cf Anden *et al* 1966a), but volleys in these afferents now produce a late long lasting DRP, which resembles that evoked on the ipsilateral side (Anden *et al* 1966a, b) The contralateral late DRP was never evoked by volleys in group I muscle afferents but could sometimes be produced from low threshold group II afferents (record "

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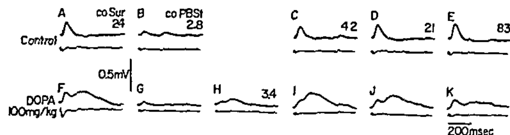


Fig 1 The effect of DOPA on the DRP evoked from the contralateral FRA The upper traces are DRPs recorded from the most caudal dorsal rootlet in L6 the lower traces are recorded from the L7 dorsal root entry zone Records A—E were obtained before and F—K after an intravenous inject on of DOPA (100 mg/kg) The contralateral sural nerve (co Sur) was stimulated in A and

same time scale for all records

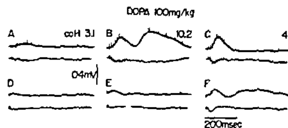


Fig 2 As in Fig 1 but recording after DOPA had been given and stimulat on of the contralateral hamstring nerve (co H)

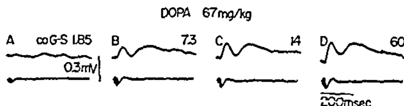


Fig 3 As in Fig 1 but recording after DOPA had been given and stimulation of the contralateral gastrocnemius-soleus nerve (co G S

although in many experiments it was necessary to stimulate group III afferents In record I Fig 1 a maximal contralateral late DRP was evoked at a strength of 4 2 times threshold and when the stimulus strength was raised there is an increase of the latency and a decrease of the magnitude (J and K) An even more pronounced reduction with increased stimulus strength is shown in Fig 2 in which a late DRP is evoked in B and abolished in C when the stimulus strength is raised from 10 2 to 41 times threshold The corresponding lower records (E and F) show that with single stimuli of the same strengths the late DRP appears when the strength is raised from 10 2 to 41 times threshold The opposite effects in B, C and E F support the assumption that volleys in the same afferents can produce and inhibit the late DRP (cf Anden *et al* 1966 b) Fig 3 illustrates an experiment in which the late

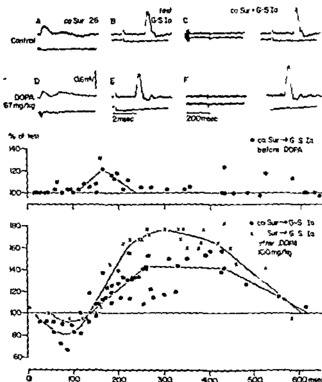


Fig 4 Measurement of the change in excitability of Ia afferent terminals by volleys in contralateral cutaneous afferents before and after DOPA The upper traces in A and D are DRPs recorded as in Fig 1, the lower traces in all records are from the L7 dorsal root entry zone The upper traces in B C, E and F show the antidromic volley recorded in the G-S nerve when its Ia afferent terminals were stimulated through a microelectrode, whose tip lay in the G-S motor nucleus For each curve the abscissa shows the interval between the first conditioning volley and the test volley, the ordinate the percentage change in the amplitude of the conditioned antidromic volley, taking the unconditioned response as 100 % A—C and the upper curve were obtained before DOPA, D—F and the lower curves after an intravenous injection of DOPA (100 mg/kg) B and E show the unconditioned test response in Ia afferents. The effect of a train of conditioning volleys in the co Sur is shown by the right hand records in C and F Conditioning testing interval is shown by the simultaneously obtained slow left hand records in C and F Observe that before DOPA there is a small late wave in the DRP and a corresponding slight increase in the Ia excitability and that these *effects increase markedly after DOPA For comparison the lower curve shows the* effect (x) of the same train of volleys in the ipsilateral sural nerve (i Sur) The voltage scale refers to the DRPs The cord dorsum record in D was taken at a higher amplification than in A The time scale for A, D and left hand records in C and F is given in F and for the other records in E

DRP did not decrease when the stimulus strength was raised to activate high threshold group III muscle afferents

Wall's (1958) technique of measuring the excitability in afferent terminals was employed to find out in which afferents the late depolarization is evoked on the contralateral side It was a consistent finding that the excitability increases in Ia afferents (flexors and extensors Fig 4 shows measurements, before and after DOPA, on Ia terminals in the G S motor nucleus In this case volleys in the contralateral sural *nerve evoke a slight late increase of the excitability even before* DOPA and correspondingly there is also a small late wave in the DRP (A) After DOPA the late wave is more pronounced and the conditioning volleys in the contralateral sural nerve now give a larger increase in the excitability which follows the initial decrease (●) For comparison is also shown the effect evoked from the ipsilateral sural nerve (×) A contralateral small late DRP and a corresponding increase in the Ia excitability was found rather often in acute spinal cats w' had not been injected with DOPA Similar findings were occasionally

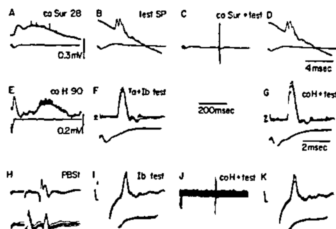


Fig 5 Measurements of the excitability of cutaneous Ia and Ib terminals when conditioned by volleys in the contralateral FRA after DOPA A and E are DRPs recorded as in Fig 1 The lower traces in all records and also the upper trace in H are from the L7 dorsal root entry zone The antidromic test discharge in B—D is recorded in the cutaneous superficial peroneal nerve (SP) and evoked from a microelectrode, whose tip lay in the dorsal horn where a maximal extracellular focal potential was recorded from the SP nerve The unconditioned test is shown in B a train of conditioning volleys in the co Sur has no effect (D) The conditioning testing interval is shown in C In E—K obtained in another experiment the tip of the intraspinal stimulating electrode was in the intermediate nucleus and the antidromic volley recorded in the PBSt nerve G shows the facilitatory effect of conditioning volleys in the co H at the same strength as in E (unconditioned group I test in F) In I—K a maximal Ia volley from the PBSt nerve is collided with the anti [illegible]

the ipsilateral side (Anden *et al* 1966b) but it is not known why without DOPA the late effect to Ia afferents is more frequently encountered in contralateral than in ipsilateral Ia afferents Crossed effects to Ia afferents from the FRA were not found by R M Eccles, Holmqvist and Voorhoeve (1964a) but this is not surprising since they worked on preparations under Nembutal anaesthesia and the late waves are completely abolished by 5 mg Nembutal i v

Fig 5 illustrates excitability measurements from Ib afferents and from cutaneous afferents B shows the unconditioned test discharge evoked by intraspinal stimulation of cutaneous afferents Conditioning volleys in a contralateral cutaneous nerve evoke a late DRP (A) but does not change the test discharge (B) F shows the unconditioned antidromic group I volley evoked from the intermediate nucleus When conditioned by volleys in high threshold muscle afferents the test discharge is increased in G This increase is due to the raised excitability in Ia afferents because when the collision technique is employed to evoke Ib test the same conditioning volley has no effect (I and K)

Discussion

In the normal acute spinal cat volleys in the FRA evoke an early contralateral DRP which is caused by a PAD in cutaneous afferents (probably in the FRA) and to some extent in Ib afferents, while there is no depolarization in Ia afferents

(R M Eccles *et al* 1964a, b) After injection of DOPA the early contralateral DRP from the FRA is depressed (Anden *et al* 1966 a) and volleys in the FRA evoke a late long lasting contralateral DRP (Fig 1—3), which resembles that recorded on the ipsilateral side (Anden *et al* 1966 b) This contralateral late DRP is caused by a depolarization in Ia afferent terminals and there was no trace of a depolarization in Ib and cutaneous afferent terminals Apparently there is a selective pathway from the FRA not only to ipsilateral (Andén *et al* 1966 b) but also to contralateral Ia afferents Since also the short latency effects from the FRA are bilateral (R M Eccles *et al* 1964 a, b) the generalizing postulate is forwarded that the effects from the FRA to primary afferents are bilateral

Evidence has been presented (Fig 1 and 2) showing that the crossed pathway from the FRA to Ia afferents is under the same inhibitory influence from the FRA as the corresponding ipsilateral pathway (Anden *et al* 1966 b) By analogy it is suggested that without DOPA also the crossed pathway is inhibited by activity in the short latency path from the FRA and that DOPA by depressing transmission in the latter path releases the long latency path from this inhibitory control

It will be shown in a forthcoming paper in this series that knowledge of the nature of the contralateral late DRP is essential for the understanding of the mechanism for reciprocal activation of flexor and extensor motoneurones from the FRA after DOPA (cf preliminary report by Jankowska *et al* 1965 Lundberg 1965 1966)

This work was supported by the Swedish Medical Council (Project No 14X 94 01 and 02 [illegible]

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Acta physiol. scand. 1966. 68. 342—346

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Monoamine Oxidase Activity in the Bovine Splenic Nerve Granule Preparation

By

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Received 6 May 1966

Abstract

ROTH, R. H. and L. STJÄRNE. *Monoamine oxidase activity in the bovine splenic nerve granule preparation.* Acta physiol. scand. 1966. 68. 342—346.

Monoamine oxidase (MAO) activity was determined in fractions obtained from bovine splenic nerve with both dopamine (DA) and noradrenaline (NA) as substrates. DA was found to be a much better substrate for MAO than NA. The bulk of the MAO in this preparation was found in the nerve granule fraction which by electron microscopic evaluation seemed to be largely free from mitochondrial contamination. These findings suggest that bovine splenic nerve in addition to mitochondrial bound MAO also contains a form of MAO with a substrate preference for DA which seems to be associated with the nerve granules.

In work currently under progress in this laboratory, DA-$^3H^3$ has been routinely used to quantitatively and qualitatively evaluate the β-hydroxylation step in the synthesis of NA in bovine splenic nerve granules (Stjärne, Lishajko and Roth, 1966). It was therefore considered desirable to also investigate the relative activity of MAO present in this preparation, since the labeled amine might be degraded to some extent by this enzyme. It has been previously assumed that little MAO activity is present in the bovine splenic nerve granule preparation since balance studies involving the release of NA from nerve granules indicated that most of the NA lost from the granules over a 1 hour period at 20° C was recovered as intact NA in the supernatant (Euler, 1961). However, it is also known that DA is a much better substrate for MAO than is NA (Weiner, 1960) and therefore MAO may play a more prominent role in regulation of the level of this monoamine in bovine splenic nerve.

The present investigation indicates that not only is there substantial MAO activity present in bovine splenic nerve but that a large portion of this activity appears to sediment with the granule fraction.

Methods

Fresh bovine splenic nerve tissue was obtained from the slaughter house, carefully dissected free from contaminating tissue, and homogenized at 0—5° C in isotonic po-

tassium phosphate pH 7 5 (10 g/30 ml), either by means of an Ultra Turrax apparatus or by squeezing between nylon rollers The whole nerve homogenate pressed juice was then centrifuged at 9 000 × g for 10 min and the pellet discarded The low speed supernatant which contains the specific NA storing nerve granule was used for the experiments In some cases the granules were separated by centrifuging the supernatant at 50 000 × g for 30 min The granules obtained were then resuspended in an equal volume of istonic potassium phosphate

One to 2 ml of the above prepared granules or supernatant – granules were incubated at 37° C in a centrifuge tube for varying time intervals and with different substrate concentrations of NA and DA In all experiments the tubes contained 1 μc of tritium labeled DA (New England Nuclear Corp, 3 4 dihydroxyphenylethyl 1 H^3 amine HBr, sp act = 50 mc/mM) or 1 5 μc of H^3 d l NA New England Nuclear Corp, purified prior to use by ion exchange column chromatography sp act = 7 c/mM) The reaction was terminated by the addition of 0 2 ml of 2 M perchloric acid and the tubes were placed in an ice bath The suspension was neutralized to about pH 6 by addition of potassium carbonate and then centrifuged at 30 000 g for 10 min at 0° C to remove the precipitated protein and perchlorate The super natant was then decanted into stoppered glass tubes containing 200 mg of Dowex 50 The tubes were shaken in a mechanical shaker for 15 min to assure quantitative adsorption of all the amines and then centrifuged at 2500 RPM for 5 min to obtain a clear supernatant One hundred μl of the supernatant which contains the deaminat ed metabolites was taken for radioactive determination Control tubes were precipitat ed with perchloric acid before addition of the radioactive substrates and then carried through the identical procedure The radioactivity was determined by counting the samples in a Packard Tri Carb liquid scintillation spectrometer The scintillation mixture consisted of a 7 3 toluene absolute ethanol solution containing 4 g of 2 5 d phenyloxazole and 100 mg of 1 4 bis [2 (4 methyl 5 phenyloxazolyl)]benzene liter of toluene Quenching was monitored by internal standards

Results

Preliminary experiments employing C^{14} tryptamine for MAO determination accord ing to the method of Wurtman and Axelrod 1963 in homogenates from bovine splenic nerve prepared by the Ultra Turrax method demonstrated that about 60 % of the MAO in this preparation was present in the low speed supernatant Further experiments were undertaken to clarify this finding

Initially experiments were run to determine how long the MAO activity remained linear When either DA or NA was used as a substrate 160 nmoles ml it was found that the MAO activity in the low speed supernatant preparation remained linear for at least 16 min A shorter time interval of 8 min was chosen to study the effects of increasing substrate concentration on the rate of deamination Fig 1 shows the results obtained with splenic nerve low speed supernatant In this preparation the MAO seemed to be saturated at a substrate concentration of 320 nmoles/ml when either DA or NA was used as a substrate The rate of deamination of NA

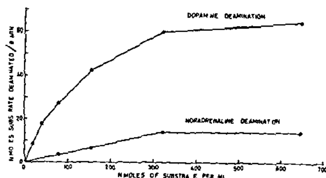


Fig 1 Nmoles of dopamine or noradrenaline deaminated by splenic nerve low speed supernatant incubated for 8 min at 37° C in the presence of various concentrations of substrate

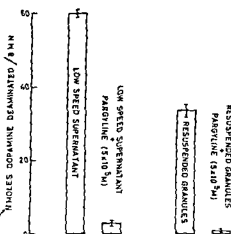
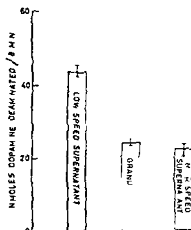


Fig 2 Monoamine oxidase activity (expressed as nmoles of dopamine deaminated/8 min) in fractions obtained from Ultra Turrax treated bovine splenic nerve both in the absence and presence of Pargyline 5×10^{-6}M Vertical bars depict the range of three determinations

strate concentration of 320 nmoles ml (i e when the enzyme appears to be saturated) was only about 1 5 of the rate of DA deamination

The resuspended granule fraction contained over 50 % of the MAO activity of the low speed supernatant (*cf* Fig 2) Pargyline (Eutonyl[R]) at a concentration of 5×10^{-6}M was found to be a potent inhibitor of the MAO activity in both systems studied At this concentration the inhibitory effectiven e c

e results are summarized in Fig 3 The granule fraction obtained by this procedure was also taken for electronmicroscopic analysis According to electron microscopic evidence (*cf* Fig 4 this preparation appears to be strikingly homogenous and apparently largely free from contamination with mitochondria The results on the distribution of MAO obtained with this pressed juice preparation are similar to those obtained with the fractions prepared by the Ultra Turrax method and again demonstrate that a large percentage of the MAO activity is bound in a particulate fraction which appears morphologically different from mitochondria



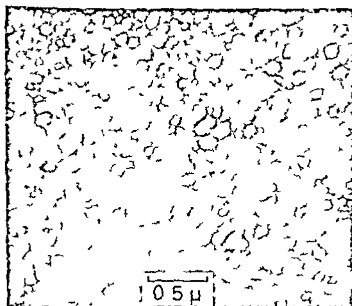


Fig 4 Electron micrograph (Euler, Stjärne, Lishajko, personal communication) of the high speed sediment obtained by centrifugation of the press juice (cf. Euler 1958) at 50 000 g for 30 min after previous removal of the coarse fraction by centrifugation at 9 000 g for 10 min

Discussion

The present experiments demonstrate that the granule fraction obtained from bovine splenic nerve contains a substantial amount of MAO activity. The MAO present shows a substrate preference for DA over NA despite the fact that DA-1-H^3 was used to measure the activity. It would be expected that some isotope effect should be apparent with this substrate (Belleau and Moran 1963) and therefore the rate

deamination estimated for DA is probably at best a low estimate of the actual rate of deamination of unlabeled DA

Blaschko, Hagen and Hagen (1957) have demonstrated that the majority of the MAO is mitochondrial bound and can be separated from the amine granules by density gradient centrifugation The electron microscopic observations on the bovine splenic nerve granule fraction, however, seems to suggest that the MAO that follows the granule fraction is not present in normal mitochondria, since these are almost completely absent in this preparation However, the possibility still remains that some sort of specialized mitochondria exist in this preparation with morphology similar to that of the amine containing granules Further investigations are now under progress in this laboratory in an attempt to relate the NA, ATP, protein, succinic dehydrogenase, β-hydroxylase and MAO content of the various fractions of bovine splenic nerve homogenates obtained by density gradient centrifugation

This investigation was supported in part by a Public Health Service fellowship 1 F2 GM 30 064 01 (R R) from the National Institute of General Medical Sciences and grants from the Swedish Medical Research Council projects No 14 X 97 02 (U S v E) and 14 X 625 01) (L S) which are hereby gratefully acknowledged

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Acta physiol. scand 1966 68 347—359

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Length at Inactivated Contractile Elements, Length-tension Diagram, Active State and Tone of Vascular Smooth Muscle

By

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Received 9 May 1966

Abstract

L. Lundholm and E Mohme Lundholm *Length at inactivated contractile elements length tension diagram, active state and tone of vascular smooth muscle* Acta physiol scand 1966 *68* 347—359

Analysis of the mechanical properties of vascular muscle is complicated by the fact that normally it is contracted to a certain degree and has no constant resting length By total blockade of the energy production of the muscle with monoiodoacetic acid under anaerobic conditions the vessel length at inactivated contractile elements could be measured With the aid of this length the length tension diagram of the vascular muscle in untreated and adrenaline treated vessels was studied The muscle developed a maximal tension (2 100 gwt/cm² cross section) at this length which therefore probably corresponded to L_0 in skeletal muscle The vascular muscle shortened maximally to ? per cent of the relaxed length Both the development of maximal isometric tension and the maximal isotonic shortening were of the same magnitude in vascular as in skeletal muscle The series elastic component (15—20 per cent) was on the other hand greater in vascular than in skeletal muscle No rigor mortis was noted in the vascular muscle The spontaneous tension of the vascular muscle and tension increase induced by adrenaline could be largely ascribed to an active state The question of whether a "tone" or "catch" mechanism is present in vascular muscle independent of the active state is discussed

In studies of the elastic and mechanical conditions of the vessel wall, certain properties are encountered that may be ascribed to its contractile elements and others that may be ascribed to the elastic and collagenous elements As in the case of skeletal muscle (Hill 1949) it may be appropriate to envisage the mechanical properties of the vessel wall schematically be assuming that it consists of a system with three components 1) the contractile element 2 the elastic component combined serially with the contractile element, 3) the elastic component running parallel with the contractile element The vascular wall has in addition marked viscous properties None of these elements or components appear to be consistently identifiable with any morp[illegible] structures in the vessel wall The contractile element obviously consists [illegible] muscle cells It is probable [illegible] smooth muscle together with

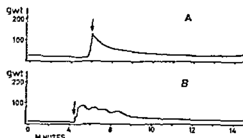


Fig 2A Tension curve for vascular specimen in which the length was quickly increased from 11 0 mm to 13 0 mm The len...

tion At the arrow the specimen was lengthened from 11 0 to 12 0 mm, whereby the tension rose to approximately 40 gwt This was followed by spontaneous rhythmic tension-increasing "stretch response"

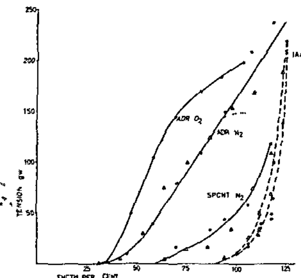


Fig 3 Length tension curves in three different specimens from the same vessel on successive extension Adrenaline (A) in a concentration of 1×10^{-6} was added and the maximal tension increase was noted The adrenaline was then washed out and the specimen was stretched 2 mm After 30 min A was again added and the procedure was repeated One ...

(○——○ and △——△) were suspended in N_2-gassed solution (ADR-N_2), the relaxed lengths were 16 0 and 14 5 mm respectively

The dotted line (ADR N_2) is the tensiondifference between A and IAA treated muscle (= active tension) Spont N_2 denotes the initial tension in anaerobic specimens before addition of ...

after A is calculated here as approximately 2,100 gwt/cm² The load at relaxed length (10 gwt) was calculated to be 130 gwt/cm²

length of the specimen between the points of attachment was 5 + 2 = 7 mm The length and tension of the specimen could be determined simultaneously in this way

The tension in the vascular specimen was measured with a Grass force transducer FTO3 and

Determination of the length tension curve

The length tension relationship was determined in untreated and adrenaline (A)-treated vascular ... contracted and ... were ... tion ... sion development after treatment with A was determined as in point (2), the specimen was extended repeatedly, by 1—2 mm at a time, before A was added It was only stretched sufficiently for the maximal momentary tension increase to reach 200 gwt When the tension had decreased and after 10—20 min, had become stable at a lower level, the vessel was again stretched until the desired length was obtained and the tension had stabilized at a new level In this way attempt was made to eliminate from the calculations the tension induced by viscous resistance of the vascular muscle on changes in length

The contraction of the vessel was induced by A in a concentration of 10 ⁵ this concentration having a maximal tension increasing effect In order that A should not be oxidized and exert a progressively diminishing effect, several experiments were performed under anaerobic conditions The maximal contraction increasing effect of A was somewhat greater in oxygen than in N_2 (Fig 3)

Muscle length at inactivated contractile elements

however, the specimen was allowed to relax overnight and its length was measured about 16 hrs after the addition of monoiodoacetic acid The length-tension curves of the specimens after 4 and 16 hrs' treatment with monoiodoacetic acid showed similar courses

The concentrations of adenosine triphosphate (ATP) and creatine phosphate (CrP) in vascular muscle were determined according to the method of Adam (1962)

Results

The concentration of energy-rich phosphate compounds in untreated and monoiodoacetic acid-treated vascular muscle Vascular muscle which had been immersed in the tissue bath for 30—60 min at 37° C in Tyrode's solution containing glucose and gassed with 93 5 % O_2 + 6 5 % CO_2, contained on the average (n = 13) 0 91 ± 0 10 μmoles ATP/g wet weight and 0 66 ± 0 09 μmoles CrP/g After treatment of the specimens with 0 022 M monoiodoacetic acid for 8—24 hours in Tyrode's solution gassed with N_2, they were found to contain neither ATP nor CrP Since with the analysis method used ATP of CrP could be demonstrated in as low concentrations as 0 02 μmoles/g, the concentration of energy-rich phosphate compounds in the moiodoacetic acid-treated specimens was reduced to below 1 5 % at least, of that in the untreated specimens

Rigor mortis in vascular muscle A striated muscle that completely loses its ATP content develops rigor mortis so that tension occurs on stretching of the muscle (Bendall 1960) We have attempted to demonstrate rigor mortis in vascular muscle, but have been unsuccessful In these experiments maximal isotonic contraction was induced by A in one vascular specimen so that its length was about 30 per cent of the relaxed length Monoiodoacetic acid was then applied to the maximally contracted specimen which was left with no load applied After 16 hours the length-tension curve was determined Tension developed at the same length as in the vascular specimen which was already extended to its relaxed length before treatment

monoiodoacetic acid No signs of rigor mortis were seen, and in this respect vascular and skeletal muscle thus seem, in principle, to behave differently

The length-tension curve in untreated and monoiodoacetic acid-treated muscle

Fig 3 illustrates a typical experiment in which the untreated vascular specimen was slowly extended by 2 0 mm at a time The tension rose considerably at first, and then rapidly decreased (Fig 2A) When the viscous resistance of this vascular tissue had been overcome, after 20—30 min, the tension become stable at a higher level, the value of which is given in Fig 3 spont-N_2 The length of the vascular tissue is given in per cent of the relaxed length (see below) With increasing length the tension rose hyperbolically

The specimen was then shortened so that the tension decreased to zero, after which it was treated with monoiodoacetic acid The length tension diagram was then determined 24 hrs later (curve IAA) The length tension diagram after monoiodoacetic acid was deviated markedly to the right, i e towards greater lengths Another difference was that as long as the monoiodoacetic acid treated specimen was only loaded with up to 50 gwt, there was no lengthening of the vessel The tension was *constant from the start, and no viscous resistance occurred* However, *when monoiodo-*acetic acid treated vascular tissue was subjected to tension exceeding 50 gwt, a lengthening occurred and a viscous resistance was noted On successive extension of the vessel the tension curves even lay above that obtained with subsequent successive shortening, i e hysteresis occurred At 10—20 gwt, however, the curves again converged on repeated extension and shortening of the vessel (Fig 7)

Muscle length at inactivated contractile elements At a tension of 10 gwt (about 100—150 gwt/cm^2 cross section) the monoiodoacetic acid treated vascular tissue therefore exhibited a constant length which remained unchanged both on successive extension *and shortening The length of the monoiodoacetic acid-treated specimen at 10 gwt* has therefore been taken as reference length in the following, it is denoted as "relaxed length", and other lengths are given in per cent of this measurement

Length-tension diagram after adrenaline Fig 3 shows also a typical experiment where vascular specimens (at different lengths) were contracted with A and the tension was related to the muscle length in per cent of the relaxed length The effect of A was first determined on the maximally contracted specimen, after which the specimen was extended successively *before each new addition of A Three different specimens* from the same vessel were studied in this experiment, two under anaerobic and one under aerobic conditions *The spontaneous tension, i e the tension before the addi*tion of A, of the two anaerobic specimens is also given in Fig 3 The difference between the curves for spontaneous tension and tension after addition of A gives the increase in tension which can be ascribed to A Also shown in Fig 3 is the length-tension diagram after treatment of the specimen with monoiodoacetic acid It is highly probable that the tension differences between the curves for "monoiodoacetic acid", on the one hand, and "spontaneous tension" and "A", on the other, can be ascribed to the contractile elements As seen in the figure, the tension increase after A was

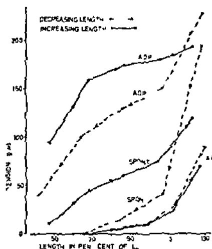


Fig 4 Length tension diagram of two specimens from the same vessel In one of them (○——→——○) the spontaneous tension and the tension after addition of A were recorded on successive extension of the specimen In the other the tension on successive shortening was recorded (○ ← ○)

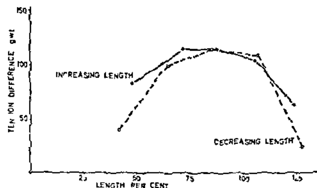


Fig 5 Difference in tension between A induced and spontaneous tension in the experiment illustrated in Fig 4

maximal at the relaxed length, when it reached a value of approximately 2 000 gwt/cm² crossection In skeletal muscle the maximal tension development is approximately 2,000—3,500 gwt/cm² Wilkie 1954 Ramsey 1960 At 25 30 per cent of the relaxed length vascular muscle is no longer exert any tension but seems to be maximally contracted In other respects the length tension curve after treatment with A followed the same course as has been described for skeletal muscle Wilkie 1954) and smooth muscle such as rabbit uterus Csapo 1960, and taenia coli (Aaberg and Axelson 1965 The maximal shortening of the vascular muscle in per cent of the relaxed length was the same as has been reported for skeletal muscle in per cent of L_0 Ramsey 1960

Fig 4 illustrates an experiment in which two different specimens from the same vessel were studied one on successive extension and the other on successive shortening The length tension curve with stepwise extension lay considerably above that with successive shortening When the tension increasing effect of A was determined in relation to the spontaneous tension, however, the increase was found to be almost the same in the two specimens Fig 5) The curves showed a level maximum at 75—100 % of the relaxed length

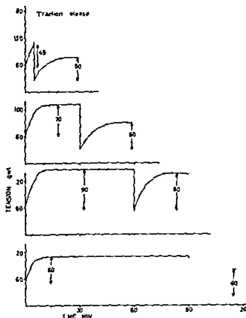


Fig 6 Active state determined by traction release Adrenaline (1 × 10 ⁸) was added to all specimens at time 0 After 6 30 60 and 90 min the specimen was extended 2 mm but so slowly that the tension increase never exceeded 200 gwt When the tension had begun to diminish the length of the vessel was rapidly adjusted The increase of active tension after 30 min was recorded The figures denote the tension increase in gwt after treatment with A before and after traction release

Determination of active state In attempts at determining the active state of vascular muscle we first tested the quick release method of Ritchie (1954) With this method the isometrically contracted muscle was shortened 1—2 mm by tightening the micrometer screw 1—2 mm within 1 second so that the tension decreased to zero These vessels had a maximal isotonic shortening velocity of approximately 1 mm min It was not necessary therefore to "release the muscle very rapidly for avoidance of active shortening The extent to which the muscle regained its original tension was then observed This procedure was usable but had certain disadvantages In vascular muscle the serie elastic component was so large that the muscle had to shorten about 2 mm (15—20 per cent of the relaxed length for the tension to decrease momentarily to 0 gwt In skeletal muscle the serie elastic component is reported by Hill (1950) to be 3—4 per cent of l At the shorter length the maximal tension development of the vascular muscle was considerably reduced *cf* Fig 3) in comparison with the greater length The muscle never therefore regained its original tension after quick release With this method the existence of an active state could be demonstrated but its intensity could not be determined In an attempt to eliminate this source of error the quick release technique was combined with preliminary extension of the muscle, and the active state was determined by traction release With this method the muscle was extended initially 1—2 mm This was done over the course of 1—2 mins and care was taken that the tension should not exceed 200 gwt (Fig 6) When the decrease in tension had begun to diminish and the tension was only a little greater than before the muscle was lengthened quick release (1—2 mm) to the original muscle length was performed With this the tension fell to a level below

the original but then rose, first rapidly and then more slowly ... a h a constant level after 10—20 mins (Fig 6) The increase in tension after ... uck release was assumed to reflect the degree of active state of the muscle

The risk with this procedure was that extension of the muscle might provoke stretch response and create an active state where none existed pre... sly H... ever it seems to be difficult to provoke a stretch response in these vessel Fig 2 A Under normal conditions the tension never rose above the level that was induced by the extension and the final tension was not reached until 20—30 min after this extension In experiments where the muscle was suspended for 24 hrs in Ca free Tyrode s solution however a stretch response was induced Extension of the muscle after this treatment induced an active tension increase which markedly exceeded that induced by extension (Fig 2 B)

Another question was whether traction release in any way reduced the reactivity of the muscle One isolated traction release did not affect the vascular muscle The contractile effect of A was the same before as after a traction release In ... is case A was washed out between the experiments With repeated traction release t the same muscle specimen in the constant presence of A however the development of acti tension successively diminished

Active state in the vascular muscle It seemed highly probable that the difference in tension between A treated and monoiodoacetic acid treated muscle could be ascribed to the contractile element But was this tension due to an active state or was it dependent on a tonic or catch mechanism in the vascular muscle? This problem was of especial interest since in previous experiments on vascular muscle we had found that on isometric contraction induced by A the energy requirement was 3—4 times greater during the phase of contraction in which tension was being increased than when a constant tension level had been reached (Lundholm and Mohme Lundholm 1965) On investigating the energy requirement on isotonic contraction of the vascular muscle we found that this requirement increased during the shortening phase and then returned to the basal value (Lundholm and Mohme Lundholm 1962 in spite of persistence of the isotonic contraction If a parallelsim was assumed between the energy requirement and the intensity of the active state these results could indicate that during the later stage the degree of active state had decreased and a catch mechanism was possibly in operation

The degree of active state with continuous influence by A is shown in Fig 6 After traction release the increase in tension from the initial value after 6 30 60 and 90 mins treatment with A was of the same magnitude as the initial tension increase on addition of A The absolute tension developed by the muscle was however lower after traction release when the initial tension lay at a lower level The results indicate however that the degree of active state was unchanged on continuous treatment with A

Another interesting question was whether the spontaneous tension noted in the muscle was a consequence of active state or was due to a more passive mechanism

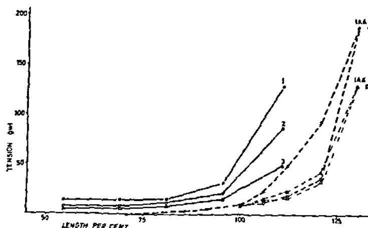


Fig 7 Spontaneous tension and active state Curve 1 spontaneous tension of the vascular specimen on successive extension by 2 mm each time Curve 2 the tension finally developed by the specimen 30 min after traction release The tension differences between (2) and (3) may be ascribed to an active state After monoiodoacetic acid treatment two consecutive length tension determinations were made (a and b)

Traction release was therefore also performed on untreated vascular muscle at different lengths in an attempt to answer this question The results are shown in Fig 7 In this figure, "1" is the initial spontaneous tension, "2 ' the tension regained by the muscle after traction-release and 3" the lowest tension exhibited immediately after release The differences between '2 ' and '3" should therefore reflect that part of the spontaneous tension that resulted from an active state As may be seen, the spontaneous tension could be ascribed partly, but not wholly, to an active state

Discussion

Relaxed length at inactivated contractile elements One important question in evaluating the signification of relaxed length was whether monoiodoacetic acid in the con centration used only influenced the metabolism and thereby the contractile state of the vascular muscle, or whether it also affected the elastic properties of the vessel wall by means of a physico-chemical process Several factors support the former idea 1) the final length attained by the muscle when loaded isotonically during treatment with monoiodoacetic acid was the same as that observed when the metabolism was inhibited by total lack of substrate 2) in taenia coli a relaxed length can be induced by total inhibition of the electrical activity of the membrane with A Monoiodoacetic acid did not cause further lengthening of adrenaline treated taenia coli Aaberg and Axelson 1965) 3) normal veins from man were relaxed by monoiodoacetic acid, varicose veins which almost completely lacked smooth muscle were not relaxed by this substance (Jonsson and Ahrenander 1963

It seems probable that monoiodoacetic acid relaxed the vascular muscle by inhibition of the metabolism, and that the length tension curve obtained here reflected the conditions in vascular muscle where the contractile elements were inactivated.

It should be possible in an inactive muscle to establish a certa[illegible] reference or resting length But, as seen in Fig 3 and 7, this length depended on th[illegible] applied to the muscle The length at a load of 10 gwt (approximately 130 gwt cm² cross section) was chosen in view of the fact that at this length and tension no hystere[illegible] occurred [illegible] repeated extension and shortening At this length the isometric tensio[illegible] was at its maximum (dotted line Fig 3) and agreed in this respect with L_0 in ske[illegible]al muscle (Ramsey 1960)

Active state If the length-tension curve after treatment with monoiodoacetic acid reflected the conditions of the inactive muscle, the vascular muscle under normal conditions is always contracted to some extent But did the contracted state of the muscle result from an active state alone? The increase in tension after treatment with A was probably due to an active state since the muscle regained its tension after traction-release The energy utilization was also increased when the muscle contracted isometrically after A (Lundholm and Mohme-Lundholm 1965

The absolute level reached by the tension after treatment with A was dependent, however, on the initial spontaneous tension of the muscle (Fig 4) The spontaneous tension and the tension resulting from A treatment appeared to be combined The spontaneous tension was, in turn, probably due in part to an active state, especially at greater lengths (Fig 7) This indicates that the active state induced by A, and the spontaneous active state of the muscle, would be added together This supposition that active state of different origin may be added is not improbable When the concentration of A in the suspension solution of the vascular muscle was successively increased until further increase in concentration gave no rise in tension, a further tension increase could be induced by histamine and/or K ions

The interesting question of whether the degree of active state in these experiments was due to different degrees of activation of the individual muscle cells or to the fact that different numbers of muscle cells were maximally activated, will be discussed in a later paper

Another question was whether the increase in tension of the contractile elements on successive extension of the vascular muscle could be ascribed to an active state alone or whether a more passive tonic or catch mechanism was a contributory cause We refer here to the tension shown by the muscle in a steady state after 30 min It is seen in Fig 4 that the tension reached by the muscle at a certain length during treatment with A was considerably higher in the experiments where the muscle was successively extended than when it was successively shortened On traction release of untreated and A treated muscle (Fig 6) the tension level before traction was higher then that finally attained by the muscle after release These experiments may indicate the presence of a catch' mechanism

The monoiodoacetic acid treated muscle showed the same tension on extension and shortening as long as the maximal tension did not exceed 50 gwt The monoiodoacetic treated vessel did thus not show viscous properties in this tension region When the series elastic elements was eliminated there was no 'catch mechanism present With higher tensions, however there appeared a viscous resistance

tension difference on extension and shortening of the monoiodoacetic vessel which was reflected in the hysteresis of the length tension curve (Fig 7)

At a higher tension than 50 gwt it is probable therefore that plastic length changes occur in the serie elastic and/or parallel elastic component of the vascular muscle tissues, which may explain some of the observed tension differences If these plastic changes can fully explain the tension differences in Fig 4 is uncertain Firstly, all specimens were subjected initially to a tension of 200 gwt which was then not exceeded, in an attempt to eliminate further plastic changes from the start Secondly, tension differences occurred on traction release of untreated muscle even in cases where the maximal tension was not greater than 50 gwt The suggestion that normal vascular muscle has some catch" mechanism can probably not be excluded, there fore Its eventually connection with the 'viscous resistance' of vascular smooth muscle is the subject of further studies

Comparison between vascular and skeletal muscle

Certain fundamental biophysical properties are common to both vascular and skeletal muscle Vascular, like skeletal muscle, is able to contract to 20—30 % of its relaxed length The length tension diagrams follow similar courses The isometric tension per cm^2 cross section which can be produced maximally by a vascular muscle is of the same order of size as in skeletal muscle Also, the energy required to produce certain tension is similar in the wto types of muscle (Beviz *et al* 1965)

There are certain essential differences, however Rigor mortis is not seen in vascular muscle on the disappearance of ATP Series elastic components is 4—5 times greater in vascular than in skeletal muscle The rate of contraction is about 100 times lower in vascular than in skeletal muscle Normal arterial muscle is always more or less contracted and in an active state On isometric contraction of a normal vascular muscle an increase occurs in the degree of active state but there is no change from a resting to an active phase, as in skeletal muscle Terms such as 'resting length and resting metabolism strictly have no significance for vascular muscle

We are indebted to the assistance of Mr Andras Beviz Miss Inga Johansson and Mr Nandor Vamos Financial support was provided by the Swedish National Association against Heart and Chest Diseases and the Swedish State Medical Research Council (U 285)

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Acta physiol. scand. 1966. 68. 360—364

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Reserpine and Gastrointestinal Mucosal Mast Cells[1]

By

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Received 10 May 1966

Abstract

RÄSÄNEN, T. and E. TASKINEN. *Reserpine and gastrointestinal mucosal mast cells.* Acta physiol. scand. 1966. *68*. 360—364.

Rats were injected intra-abdominally with 2 mg/kg of reserpine once, 5 times and 9 times at 12-hr intervals. 4 hrs after the last injection the rats were killed and the mucosal mast cells of the stomach

Reserpine does not appear to cause changes in the peritoneal mast cells of the rat (Bhattacharya and Lewis 1956, Padawer 1957), thought the 5-hydroxytryptamine content of the mast cells decreases (Moran and Westerholm 1963). Nor does reserpine cause morphologic changes in the mast cells of the subcutaneous connective tissue of the rat although their serotonin content especially declines (Parratt and West 1957).

The mast cell profiles of human gastric mucosa concur with the mucosal histamine and serotonin densities. The ratio between the concentrations of these amines in the human gastric mucosa is 13:1 (Murray, Wyllis 1964) and in the rat 15/1 (Nikodijevic and Trajkov 1963), the affinity of serotonin for heparin is considerably *smaller than that of histamine (Selye 1965). For the rat skin the ratio between the* histamine and serotonin concentrations is about 50, but in the wall of the gastrointestinal canal it varies from 4 to 8 (Talfort and Eest 1960).

Mast cell granules store and synthetise not only polysaccharides but also histamine and serotonin. The amount of serotonin decreases in the skin (Parratt and West 1957). Total release of serotonin happens in a mast cell population with a 10^{-8} M concentration of reserpine (Carlini, Fischer and Giarman 1964).

The mucosal serotonin content appears to remain unchanged in the gastric mucosa of the cat during reserpinisation whereas secretion increases (Emås 1964). As

[1] Aided by a grant from the Sigrid Juselius Foundation

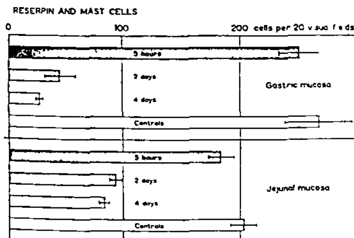


Fig 1 The columns in the diagram indicate the count of mast cells per 20 visual fields the gastric body and in the jejunal mucosa of rats

earlier studies have shown that rat gastric secretion during Shay ligature is affected by the different phases of the granulation of mucosal mast cells (Haikonen and Rasanen 1965), the authors decided to study the reactions of the mucosal mast cells during the reserpinisation of rats

Method

Male rats of Dawley Sprague strain, aged about 5 months, were used in the study They were kept in the laboratory on ordinary mixed rat diet and water ad libitum The rats were injected with 2 mg/kg of reserpine (Serpasil, Ciba) once 5 times and 9 times at intervals of 12 hours The injection was given intra abdominally

The first group consisted of rats mean weight 332 g the second of 7 rats of which one died, mean weight 269 g and the third of 8 rats of which 3 died mean weight 221 g The controls, 7 rats were given no injections Their mean weight was 357 g

4 hrs after the last injection the rats were bled under light ether narcosis Immediately afterwards, samples were taken from the gastric and jejunal mucosa mounted on a small piece of cardboard and fixed in fresh 4 % basic lead acetate After fixation sections of 10 μ in thickness were cut vertically to the surface of the gastric mucosa and stained in 1 % toluidine blue aqueous solution The mast cell count was performed in the superficial part of the gastric body mucosa and the mean height of the jejunal mucosa from the zone richest in mast cells per 20 visual fields by means of oil immersion

Results

Reserpine did not seem to cause changes in the gastric and duodenal mucosal mast cell amounts 4 hrs after its administration (Fig 1) After 5 injections over a period more than 2 days (52 hrs) the number of gastric mucosal mast cells fell by 83 per



Fig 2



Fig 3

Fig 2 Loss of metachromatic material from the mucosal mast cells of *gastric mucosa after four* days reserpinisation of rat Magnification 300 ×

Fig 3 Submucosal connective tissue mast cells in the gastric wall of reserpinised rat The mast cells contain profusely metachromatic granules Magnification 800 ×

($P < 0.001$), decreasing further in the course of over 4 days (76 hrs) to 10 per cent of the control value ($P < 0.001$) (Fig 2) For the mast cells of jejunal mucosa the comparable decreases ranged from 50 to 60 per cent ($P < 0.001$)

Unlike the mucosal mast cells in the stomach, the mast cells of the submucosal connective tissue of the rats injected with reserpine displayed no features suggestive of degranulation even after 4 days (Fig 3) The rats were almost immobile after the reserpine injection and became increasingly exhausted as the reserpinisation continued, they had no appetite, and one rat in the 2-day group and 3 in the 4 day group died The stomachs of the rats given reserpine contained bloody gastric secretion, with the exception of one case in the 4-day group

Discussion

Unlike the mast cells of the connective tissue (Parratt and West 1957) and peritoneal mast cells (Bhattacharya and Lewis 1956, Padawer 1957), in the gastric mucosal mast cells especially, but also in the mast cells of the jejunal mucosa, reserpine provokes degranulation Gastric mucosal mast cells seam to be extremely prone to lose their metachromatic material under the action of glucocorticoids, ACTH, acute stress and irradiation (Rasanen 1961, 1963) It is possible that the amount of heparin mobilised in the urine during postoperative stress, which is four times the normal quantity (Mestel, Neuhof and Rosenfeld 1965), is mobilised chiefly from the liberal number of mast cells in the gastric mucosa

Reserpine appears to be one of the most effective 5-hydroxytryptamine releasers in the central nervous system, the effect is, however, inhibited by monoamino-

oxidase inhibitors Reserpine temporarily nullifies the ability of [illegible] ain tissue to store serotonin Gastric mucosa has an about 8—10 times higher serotonin concentration than the brain and the rat gastric mucosa is capable of storing a manifold quantity of exogenous serotonin without any changes in the histamine concentrations Nike djevic and Trajkov 1963)

The formation of endogenous serotonin in the gastric mucosa is probab[illegible] a h[illegible] el process as the turnover time of radioactive serotonin in the rabbitt is shor[illegible]st in the gastric mucosa (10 hrs), 17 hrs in the intestinal mucosa and 33 hrs in the spleen and in the platelets (Udenfriend and Weissbach 1958) The sharp increase in the serotonin concentration which occurs in portal blood under shock may be caused by the argenttafine granules of Kultschitzky's cells or by platelets [illegible] ark Hissen and Fellman 1964) and also by the serotonin which is released from [illegible] ast cell of the gastrointestinal canal

In addition to histamine, serotonin is probably released from the d[illegible] nul[illegible] mast cells of the gastric mucosa under reserpine action Serotonin ha[illegible] a affinity than histamine for heparin Serotonin provokes mucosal lesions ([illegible] ke d and Trajkov 1963), like reserpine, the ulcerative effect of the latter however inhibited by monoamine-oxidase inhibitors or catecholamines (Necina and Krej 1963)

Reserpine stimulates gastric secretion independently of the autonomous nervous system (Emås 1964) Histamine disappears from the gastric mucosa in the course of the glucocorticoid effect when the mast cells of the gastric mucosa are degranulated (Foley and Glick 1962) Depending on the phase of granulation gastric secretion is stimulated or inhibited after the application of pyloric ligature in the rat Haikonen and Rasanen 1965) Reserpine induced degranulation of gastric mucosal mast cells is probably accompanied by histamine liberation and parenchymal stimulation Local liberation of serotonin may result in postcapillary spasm with mucosal hemorrhages

Reserpine provoked degranulation of mucosal mast cells may be due to its direct effect or may be exercised by the hypophysial — adrenocortical route which leads to the degranulation of mucosal mast cells (Rasanen 1961) In the rat, administered twice in a dosage of 10 mg/kg reserpine has a rather slightly increasing effect on the plasma cortisone content compared with ACTH Consequently it is probable that the degranulation demonstrated in the present study is due to a great extent to the direct effect of reserpine on the mucosal mast cells

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Cat Retinal Ganglion Cell Responses to Changing Light Intensities: Sinusoidal Modulation in the Time Domain

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Received 25 May 1966

Abstract

Cleland, B and C Enroth-Cugell *Cat retinal ganglion cell responses to changing light intensities Sinusoidal modulation in the time domain* Acta physiol scand 1966 *68* 365—381

Action potentials were recorded from single axons of retinal ganglion cells in adult lightly anesthetized paralyzed cats (intact eye) A small sinusoidally time modulated stimulus was located in the most responsive portion of the receptive field center The mean discharge frequency was monitored on a counter during the experiment and the responses over extended periods of time were recorded on tape and later averaged Most of the cells were outside area centralis A series of experiments the [illegible]
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modulation depth at constant frequency (b) a function of stimulus frequency at constant depth of modulation Only two of 31 cells exhibited (up to 40 per cent modulation) a mean discharge frequency independent of modulation depth a response amplitude reasonably proportional to modulation depth an almost constant stimulus response phase difference and an averaged response whose shape was almost sinusoidal The remaining cells failed to fulfill at least one of these requirements for linearity For 6 cells modulation sensitivity curves were determined and found to have the same general shape as those subjectively determined in humans

In a study of subjective flicker and fusion using sinusoidally time varying stimuli, deLange (1957) showed that under certain conditions the human visual system may be considered to operate linearly The behavior was found to be linear under threshold conditions at fusion whereas deLange's experiments suggested that under conditions of marked flicker at low frequencies the response was definitely nonlinear His work clearly demonstrated that linear filter theory — when applicable — is a useful tool in the study of visual systems However a linear approach to electrophysiologically recorded visual signals can be justified only if linearity of the visual processes involved is experimentally demonstrated Linearity, if established and

threshold conditions in *psychophysical* experiments, does not permit conclusions regarding the nature of *neurophysiological* stimulus response relationships under threshold or suprathreshold conditions

Several investigators have employed sinusoidal stimuli in neurophysiological experiments on invertebrate and vertebrate eyes (Van der Tweel 1961, Gouras and Gunkel 1962, deVoe 1963, Kuiper and Leutscher-Hazelhoff 1965, Hughes and Maffei 1964, Maffei, Moruzzi and Rizzolotti 1965, Pinter 1966) Retinal action potentials were recorded by deVoe (1963) from the light adapted eye of the Wolf Spider in response to sinusoidally time varying stimuli He found a mixture of both linear and nonlinear suprathreshold behavior, the response amplitude was proportional to the depth of modulation at constant frequency of stimulation and the stimulus response phase difference was independent of the depth of the modulation, as required for a linear system However, the waveform of the response showed negligible deviation from a sinusoid at only low depths of modulation, above 8 per cent the waveform of the response showed definite nonlinearities The response characteristics of cat retinal ganglion cells during stimulation with sinusoidally modulated lights have been briefly reported by Hughes and Maffei (1964), Maffei *et al* (1965) and Rackenspenger *et al* (1965)

The aim of the experiments presented here was to determine the degree of linearity of retinal signal generation and transmission and to observe the extent of nonlinearity of the retinal ganglion cells Although we realize that all parts of the receptive field contribute to the characteristics of the signal that travels in a retinal ganglion cell axon we elected first to study only responses from the receptive field center *By restricting ourselves to stimuli that involved the receptive field center* only we became acutely aware of the very real problem of maintaining prolonged, effective immobilization of the eye Without absolute stability of the globe it is impossible to obtain any quantitative measure of the steady state response to a small stimulus We are reporting our experience with this problem as well as our findings regarding the response characteristics of cat retinal ganglion cells at supra threshold and at "threshold levels during sinusoidal stimulation

Methods

a *Preparation and recording*

Adult anesthetized paralyzed and artificially respirated cats were used (For drug details see below — ' Immobilization of the eye The head was held in a stereotaxic instrument and a rectangular piece of bone (from *Horsley Clarke F to F 16 and LL 2 to LI 10)* was removed from the top of the sk ll providing access to the chiasm and that part of the optic tract over which there is no lateral

b Stimulation

For most experiments a low frequency light modulator (Gubisch 1965) was us d to project a circular stimulus onto the middle of a rectangular white, cardboard screen placed one meter from the cat The screen subtended 60 × 70 deg at the cat s eye and was evenly illuminated at 15 millilamber s To obtain the desired luminance variation with respect to time the modulator was driven by function generator in a sinusoidal or square wave fashion The maximum mean lun nance of t stimulus was 100 millilamberts Its size could be changed without changing the m luminanc The diameter of the smallest spot subtended 0 25 deg at the cat s eye The modulation depth f th stimulus luminance was variable from zero to hundred per cent

The stimulus spot was less than one deg in all the experiments As a rule the receptive field center sizes were not mapped and the stimulus and center diameter can therefore not be compared in each case However, the stimulus was always such that when placed centrally in the receptive field and modulated 100 per cent at 0 5 cps in a square wave fashion no audible impulse frequency increase could be detected at offset of light for on center cells and vice versa for off ce ter cells

In a few experiments a second stimulator was used This consisted of six fluor scen tubes en closed in a box behind opal glass located behind a central hole in a cardboard e identical to the one above) The function generator was used to vary the light intensity T sufficient range over which the light output of these tubes is proportional to the current g them to permit luminance modulation up to a maximum of 90 per cent The stimulus dian was varied by using cardboard screens with different sized apertures The maximum mean lum e was 700 m ll lamberts which was decreased with neutral density filters inserted between the n and the l ght source The front of the screen was evenly illuminated at 1 millilambert

Modulation depth m expressed in per cent is defined as $(L_{max} - L_{min})/(L_{max} + L$ wher L is the stimulus luminance As mentioned above the stimulus spot provided by the fir st ulat r was superimposed on a non modulated background of 15 millilamberts It should be not ll the numerical values given in the text and illustrations are corrected to give the true depth of modul tion as observed by the cat (i e corrected for background) With the second stimulator this complica tion d d not arise since in that case the stimulus spot was not superimposed on a non modulated background Stimulus and background strengths are given in terms of retinal illumination With fully dilated pupil (14 mm diam) 1 millilambert corresponds to approximately 300 trolands with a 3 mm d ameter artificial pupil approximately 40 trolands

c Data Processing

At some time following the experiment the tapes were played back and the data pr cessed The ganglion cell spikes were used to trigger a pulse shaper which gave a current pulse of 0 5 msec dura t on The pulses were fed to a capacitor which discharged through a fixed resistor The time constant of th s RC smoothing network was 10 msec The voltage across the capacitor was applied to one of the inputs of a digital memory oscilloscope (Enhancetron 1024 which formed the sum of a number of response cycles This sum was scaled to give the average variation in impulse density of the retinal ganglion cell discharge The stimulus was taken from the tape to the other Enhancetron input A sweep duration equal to two stimulus cycles was chosen on the Enhancetron and generally 40 sweeps were averaged Both averages were written out on an x y plotter Response amplitudes and phase differences were measured on the x y record Response amplitude refers to the peak peak amplitude of the impulse density fluctuations occurring in synchrony with the luminance modulati n F r on center cells the phase differences were measured from stimulus peak to resp nse peak for off center cells from stimulus trough to response peak (see Fig 6 and 7

Using an RC network for smoothing necessarily introduces a frequency dependent phase shift and attenuation The actual am unt of phase shift introduced by our equipment was experimentally determined at 4 8 and 16 cps and found to be 15 27 and 50 deg respectively

Results

1 Immobilization of the eye

To collect data for curves relating the mean discharge frequency of the cell and the response amplitude to the depth of modulation or for plots of response amplitude versus frequency of stimulation at least two hours of unfailing immobilization of the eye was needed Each time the depth of modulation or the frequency of the stimulus was changed the response from the cell was allowed to settle down to a new stead value before impulse train samples were recorded on the tape During the cat experiments barbiturate narcos

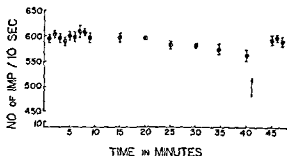


Fig 1 Mean discharge rate of an on center cell responding to a stimulus modulated 50 per cent at 8 cps The stimulus which subtended 0 5 deg at the cat s eye was [illegible] the average count over one preceding minute The length of the vertical bars is two standard deviations The position of the stimulus was adjusted at the arrow

doses commonly used by others in similar experiments Mostly doses below 15 mg/kg/hr of succinylcholine chloride or gallamine triethiodide have been used (Wiesel 1960, Bishop, Kozak and Vakkur 1962, Burns and Pritchard 1964, Kozak, Rodieck and Bishop 1964, Levick and Williams 1964, Ogawa, Bishop and Levick 1966) In one case (Rodieck and Stone 1965a) 20—25 mg/kg/hr of gallamine was reported The paralysis that we achieved with such dose rates was far from satisfactory for our purposes We were interested in quantitative aspects of the steady state output from the ganglion cells in response to a small constant stimulus Whether the quantity measured is the mean spike frequency or the response amplitude, results are reliable only if one can trust that observed changes in the magnitude of the output are due to changing the frequency and/or depth of modulation of the stimulus not to a relative image motion within the receptive field To learn how to achieve long lasting eye immobilization in the face of light anesthesia a series of stability experiments were conducted on 52 cells (22 on center and 30 off center cells) in 17 cats Light anesthesia is highly desirable in order to minimize the effect of the anesthetic upon the retinal function (Barlow, Hill and Levick 1964)

The procedure was as follows when the receptive field had been located the stimulus size was decreased to the minimum at which a good response was heard By moving the stimulus around within the receptive field center the position which yielded the highest mean impulse frequency (as noted on the electronic counter) was selected and the stimulus was left in that position When after 15—30 sec the count had seemingly settled down to a steady state the number of impulses per 10 sec interval was observed and plotted versus time

Fig 1 illustrates the effect upon the mean spike frequency of an on center cell of a slow drift of the eye The stimulus diameter subtended 0 5 deg at the cat s eye During the first 15 min the output from this ganglion cell was quite steady but then a slow decline of the mean frequency started At 40 min the counting was interrupted and the stimulus was moved to various locations within the receptive field center in search for one that yielded a higher count In this particular case a 0 5 deg movement nasally brought the mean frequency very close to the level at which it had been 30 min earlier Note that the instability in the impulse count illustrated in Fig 1 occurred while the gallamine triethiodide dose was 40 mg/kg/hr

When such slow changes in the impulse counts occurred it was possible (with one exception, see below) to bring back the count to its original value by careful readjustment of the image within the receptive field This suggested to us that the changes in the mean discharge frequency of the response to a constant stimulus were due to slow drifts of the eyes The stimulus movements required varied from a few minutes of arc to more than 1 deg in the extreme cases It might be argued that the extended exposure of a particular region of the receptive field to the sinusoidally modulated stimulus resulted in a "local fatigue" and that when the stimulus was moved, involvement of a "fresh" receptive field area resulted in increased impulse frequency We do not believe that this is so because (1) An increased dose rate of the paralyzing agent often turned a spike count of the type shown in Fig 1 into an hour long stable mean discharge frequency (2) If adjustments as large as 1 deg or more of the stimulus position increased the impulse count, because a new "non fatigued" area of the receptive field got involved, and not because the stimulus was brought back to the middle of the center, then one would have to assume that there were two rather widely separated locations within the receptive field which both responded maximally to the identical restricted sinusoidal stimulus This is highly unlikely in view of what at present is known about the sensitivity distribution within the receptive fields of cat retinal ganglion cells (Kuffler 1953, Rodieck and Stone 1965b, Enroth Cugell and Robson 1966) [1]

[1] One experiment constituted an exception to the rule that slowly decreasing spike counts could be restored by m[illegible] from 6 cells For all ce[illegible] th[illegible] ce[illegible] ca[illegible] 9[illegible]

In our earliest experiments in this series thiopentol or pentobarbitone sodium were combined with continuous intravenous infusion of succinylcholine chloride When the narcosis was light (as judged by lively retinal reactions to stimuli throughout the experiment and slight general motor unrest upon removal of paralysis) good eye immobilization was not obtained until the succinylcholine doses reached such levels that many cats died early in the day of what clinically appeared as lung edema Much better general condition of the animal was achieved with thiopentol or pentobarbitone in conjunction with gallamine triethiodide in high doses (up to 60 mg/kg hr) However, the pronounced effects of even small variations in barbiturate doses upon retinal activity (*e g* Barlow *et al* 1964) were quite disturbing Moreover pentobarbitone particularly resulted in an annoying "spontaneous rhythmicity of the ganglion cell discharge as described in rats by Brown and Rojas 1965) In later experiments barbiturates were therefore avoided for maintenance of anesthesia and urethane chloralose was used (Anesthesia was induced with diethyl ether followed by about 5 mg/kg of thiamyl sodium intravenously) In view of Berglund Nylén and Wallentin s (1965) findings on cats that the *combination* of urethane and chloralose is particularly apt to result in acidosis the chloralose was abandoned and urethane

[1] See note 1 added in proof

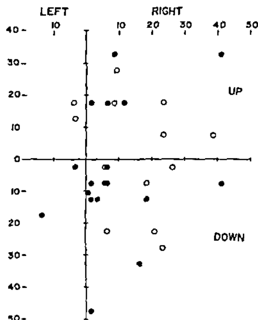


Fig 2 Approximate positions in the visual field of receptive field projections Open circles on center cells filled circles off center cells The axes are marked in angular distance (deg) from the projection of the center of the area centralis

alone used Light anesthesia was maintained with doses of 3—5 mg/kg/hr Before administration of gallamine, atropine and phenylephrine slight motor unrest was present and the pupils reacted well to light, directly and indirectly The retinal responses were brisk throughout the experiment and when after 8—10 hours the gallamine infusion was discontinued spontaneous breathing and some motor unrest returned In the cases where no atropine or phenylephrine had been instilled the pupillary reactions to light were normal

Our results from this series of experiments may be summarized as follows if a stimulus, which is smaller than the receptive field center is modulated sinusoidally about a constant mean luminance at a constant frequency and at a constant depth the resulting mean spike frequency stays quite stable (coefficient of variation $\pm$ 3 %) provided no eye movements occur and the preparation is in good general condition Unless one resorts to deep anesthesia, it is our experience that doses of 25—60 mg/kg/hr gallamine triethiodide are needed to achieve reliable long lasting immobilization of the eyes These doses are much larger than usually reported High succinyl choline chloride doses seem to be less well tolerated than high gallamine triethiodide doses

2 *Response characteristics of retinal ganglion cells to stimuli of different modulation depths and frequencies*

In a second series of experiments the response characteristics of retinal ganglion cells were studied in ten cats (13 on center and 18 off center cells) The position of the projection in space of the receptive fields of these cells relative to the projection of area centralis is shown in Fig 2 The angles were estimated to within 5 deg and cor-

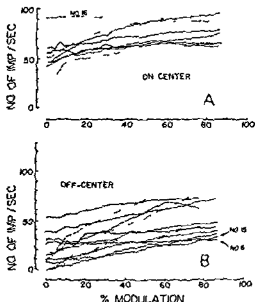


Fig 3 Mean discharge frequency of 8 on center (A) and 12 off center (B) cells in response to sinusoidally modulated stimuli at 8 cps The depth of modulation varied Full lines stimulus mean 5 × 10⁴ trolands background 7 5 × 10³ trolands Broken lines stimulus mean 6 × 10⁴ trolands background 5 × 10⁴ trolands The stimulus was smaller than the receptive field center and located in its most sensitive part

rected for the direction of the optic axes in cats paralyzed with succinylcholine chloride (Vakkur, Kozak and Bishop 1963) Clearly only a minor portion of our units were central ones

As in the previous set of experiments a small sinusoidally time modulated stimulus was positioned in the most responsive part of the receptive field center Two main types of experiments were performed (a) the frequency of the luminance modulation was held constant while the depth of modulation was varied (b) the depth of modulation was constant, the frequency of modulation was varied The mean discharge frequency of the cell, the response amplitude and the stimulus response phase difference were studied in (a) as a function of modulation depth and in (b) as a function of modulation frequency In a third type of test the experimenter determined the depth of modulation required at different frequencies to evoke a barely discernable response to the sinusoidal stimulus, i e threshold curves similar to deLange's (1957) attenuation characteristics of the human visual system were determined for a few retinal ganglion cells

a *Constant frequency of modulation*

The output of a linear system in response to a sinusoidal input of constant frequency is characterized by (1) a mean value that is independent of the modulation depth of the input (2) a stimulus response phase difference independent of variations of input modulation (3) a response amplitude which is proportional to the depth of the input modulation and (4) a waveform which is a replica of the sinusoidal input To assess the extent of linear and nonlinear features of retinal ganglion cell resp

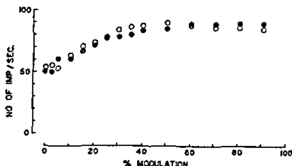


Fig 4 Two determinations of mean impulse frequency as a function of modulation depth for the same on center cell The stimulus frequency was 8 cps Open circles the first determination Filled circles the second determination which started approximately 15 min after the first one was completed

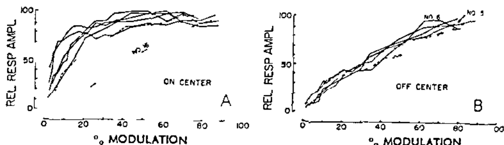


Fig 5 Relative response amplitude as a function of modulation depth Stimulus frequency 8 cps A on center cells B off center cells Full lines stimulus mean 5×10^4 trolands background 7.5×10^2 trolands Broken lines Stimulus mean 6×10^4 trolands background 5×10^2 trolands The stimulus was smaller than the receptive field center and located in its most sensitive part

the four above mentioned characteristics of the cell discharges were studied during constant frequency stimulation and the results are reported below In the majority of these experiments the stimulus frequency was 8 cps This particular frequency was selected because our preliminary experiments with sinusoidal stimuli showed that the retinal ganglion cells were most sensitive at stimulus frequencies around 8 cps This frequency falls within the region of maximum sensitivity of deLange's human attenuation curves (1957) as well as within the range of maximal mean discharge frequency for cat retinal ganglion cells stimulated with diffuse light modulated at 100 per cent in a square wave fashion (Grusser and Reidemeister 1959)

Mean spike frequency In Fig 3 the mean spike frequency recorded from 8 on center and 12 off center cells is plotted as a function of modulation depth The experimental procedure was to start with the deepest modulation and then decrease it in small steps For each new depth of modulation the number of pulses per 10 sec interval was observed on the electronic counter until the value was stable The average of three 10 sec counts was then noted (The response was also recorded on tape and later processed to yield the response amplitude for each depth of modulation) When the spike count for zero per cent had been obtained the modulation was again set at the highest value and the entire procedure repeated The two curves in Fig 4 each represent one full run from highest to lowest depth at modulation This is a

representative sample of the repeatability (provided eye immobilization is reliable) of results obtained in the type of experiments where mean discharge frequency and/or response amplitude was studied as a function of modulation depth and/or modulation frequency By checking in random order a few points on the curves in Fig 3—5 and 9 it was established that their shape was not influenced by the experimental procedure Eight of the curves in Fig 3 represent a single run from highest to zero per cent modulation, the others are averages of two or three runs Note that only one on center cell (no 16) and one off center cell (no 6) maintained a constant mean discharge frequency with changing modulation depth, at a constant frequency of stimulation In the case of all the other cells the mean discharge rate depended upon the depth of modulation, although not in the same fashion for all cells

For some cells data regarding the mean discharge frequency of the cell as a function of modulation depth were obtained at more than one constant stimulus frequency The on center cell (no 16) whose stable mean discharge rate during an 8 cps stimulus is shown in Fig 3 A, was also tested at 4 and 15 cps, both these curves were as "flat" as the 8 cps curve The off center cell (no 6) with constant mean discharge frequency at a stimulus frequency of 8 cps was not tested at any other frequencies A total of 7 cells (3 on, 4 off center cells) with sloping mean discharge frequency curves were stimulated at several constant frequencies of modulation (ranging from 0 5—28 cps) The mean discharge rate rose with increasing modulation depth at all frequencies which yielded a measurable response amplitude when the data were averaged, *i e* when the cell responded with an impulse frequency modulation

All the curves in Fig 3 were obtained with much the same retinal illumination (5×10^4 or 6×10^4 trolands) It is reasonable to question the effect of different mean values of the stimulus luminance upon the average discharge rate of the cell as a function of modulation depth Unfortunately we have very little experimental results at varying retinal illumination levels of the stimulus However two on center and two off center cells were studied (at 8 cps) at 5×10^4 and 5×10^3 trolands while the background was 5×10^2 trolands Only with stimuli of the higher mean retinal illumination was there a significant rise in the mean discharge frequency of these cells as the modulation depth increased Possibly the manner in which the mean discharge rate of retinal ganglion cells depends upon the modulation depth of a constant frequency stimulus, is influenced by the mean retinal illumination of the stimulus

Relative response amplitude Fig 5 shows relative response amplitude at 8 cps as a function of modulation depth for seven of the on center cells and seven of the off center cells of Fig 3 The magnitude plotted is the peak peak response amplitude as measured on the x y record (see Fig 6 and Fig 7, second trace from above) The curves have been normalized for 100 per cent The response amplitude of on center cells increase much more rapidly at low depth of modulation than do off-center amplitudes The on-center curves, with one exception, begin to

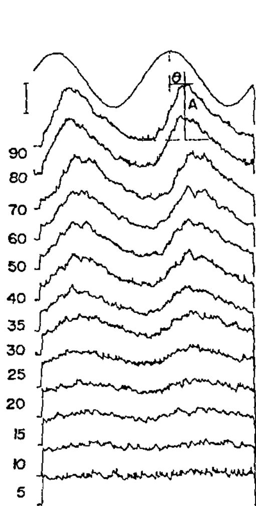


Fig 6

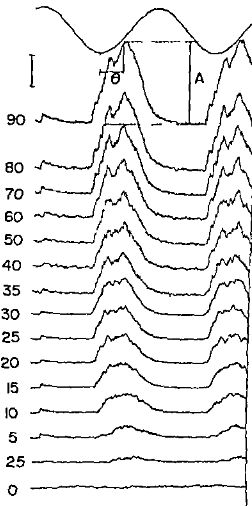


Fig 7

Fig 6 Averaged impulse discharge from one on-center cell (No 16) in response to sinusoidally modulated stimuli at 8 cps The top trace indicates the stimulus the others are the output from the cell at varying depths of stimulus modulation The number to the left of each trace is the per cent modulation The height of the vertical bar in the upper left corner corresponds to 100 imp/sec Zero imp/sec is indicated by the initial short horizontal portion of each trace The angle θ between the stimulus peak and the response peak is the phase difference The distance A between the minimum and the maximum impulse frequency during each cycle is the response amplitude Note that although the response amplitude increases with increasing modulation depth the mean discharge frequency for this cell is approximately 90 imp/sec in all tracings Stimulus mean 6×10^4 trolands background 5×10^3 trolands

Fig 7 Averaged impulse discharge from an off-center cell responding to sinusoidally modulated stimuli The top trace indicates the 8 cps stimulus the other traces are the output from the cell

between the minimum and the maximum impulse frequency during each cycle is the response amplitude. Stimulus mean 6×10^4 trolands background 5 10^3 trolands

approximately 20 per cent while the off-center curves tend to continue to rise with rather little change of slope far beyond 20 per cent modulation The response amplitude curve of the on-center cell (Fig 3A) which exhibited a flat mean discharge frequency versus modulation curve (no 16) is of a rather different character than its fellow curves for this one on-center cell the amplitude of the response increased in a nearly linear fashion (at 8 cps) with increasing depth of modulation

Waveform of the response In Fig 6 and 7 are shown the averaged responses from one on and one off-center cell during stimulation at constant frequency but changing depth of modulation In the case of this on-center cell the waveform is approximately sinusoidal up to 30—35 per cent modulation This is the same on-center cell (no 16) whose mean impulse frequency was independent of depth of modulation Fig 3A) and whose relative response amplitude at 8 cps was almost linearly related to the modulation depth (Fig 5A)

For the off center cell shown in Fig 7 (at 8 cps) the waveform of the response is clearly non sinusoidal even at the lowest depths of modulation Not only does the response appear rectified at all depths but at higher modulation levels there is in addition a double peak This was not the case for all off-center cells The mean spike frequency and the response amplitude versus modulation curves marked no 15 in Figs 3B and 5B are from an off-center cell where the waveform of the impulse frequency modulation (at 8 cps) was an almost perfect sinusoid up to 40 per cent modulation However, the off center cell responses tended to exhibit more pronounced deviations from sinusoidal shape at lower levels of modulation than did the on-center cells Under our stimulus conditions gross distortion of the waveform became apparent at modulation depths varying from 35 to 70 per cent for seven on-center cells while the corresponding figures for seven off center cells was 25 to 40 per cent

Stimulus response phase relations The phase difference between stimulus and response was measured as described in Methods and as indicated in Fig 6 and 7 for on and off-center cells respectively At 8 cps and deep modulation both types of cell exhibited an approximately 70 deg phase lag Fig 8 As the depth of the modulation decreased the phase lag increased more so for on-center cells than for off center cells The phase shift introduced by the data processing system at 8 cps amounts to 27 deg see p 367 The phase angles plotted in Fig 8 include this phase shift contributed by the data processing

b Constant depth of modulation

In this section some characteristics of retinal ganglion cell responses as a function of frequency will be described As before, the stimulus was located in the most responsive part of the receptive field center The experimental procedure for obtaining curves, which relate mean impulse frequency response amplitude and stimulus response phase difference to stimulus frequency was similar to the one used in the previous section The only difference was that in these experiments the stimulus parameter being varied was the frequency of modulation not its depth

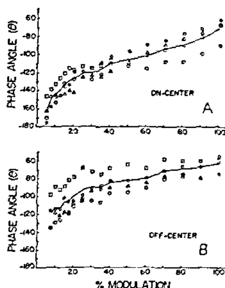


Fig 8

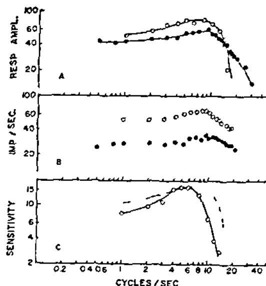


Fig 9

Fig 8 Graph of phase shift θ (see Figs 6 and 7) as a function of stimulus modulation depth (stimulus frequency 8 cps) A four off center cells B four on-center cells Different symbols represent

[illegible]

scaling is arbitrary

B mean impulse frequency for the on center (open circles) and the off center (filled circles) cell in A Modulation depth 50 per cent

C modulation sensitivity curve for the same on center cell as in A and B For each frequency the experimenter determined the depth of modulation at which he could barely hear an impulse frequency modulation synchronous with the luminance fluctuations The inverse of these values are plotted (open circles The broken line is the frequency response curve for the same cell (shown in A)

Stimulus mean in A B and C 5×10^4 trolands background 7.5×10^3 trolands

Mean spike frequency and response amplitude Frequency response curves were successfully completed for 10 ganglion cells (four on- and six off-center cells) The stimulus mean was 5×10^4 trolands (background 5×10^2 trolands) except for one on- and one off-center cell where it was 4×10^3 trolands (background 40 trolands) All the frequency response curves obtained at modulation depths higher than 15 per cent exhibited one frequency at which the response was at a maximum, falling off both at lower and at higher frequencies Similarly the mean discharge rate of the cell reached a maximum at some intermediate frequency of stimulation Fig 9 shows representative frequency characteristics for one on- and one off-center cell A and B are plots of the response amplitudes and the average impulse discharge frequencies at 50 per cent modulation. The curve in Fig 9C represents an auditory determination of the smallest modulation depth required at each individual frequency for the experimenter to barely detect an impulse frequency fluctuation synchronous with the stimulus modulation The values plotted are the inverse of this "threshold"

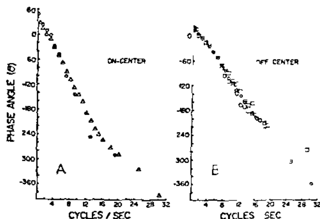


Fig 10 Graph of phase shift θ (see Fig 6 and 7) as a function of stimulus frequency Depth of modulation 50 per cent A two on-center cells B three off center cells Stimulus mean 5×10^4 trolands background 7.5×10^3 trolands

modulation No such sensitivity curve was determined for the off center cell shown in Fig 9 Modulation sensitivity curves were determined for three on and two off center cells and they all had the general character of the curve shown in Fig 9 i e a maximum at some intermediate frequency of stimulation

Stimulus response phase relationships Fig 10 shows representative plots of the phase shift as a function of stimulus frequency for two on and three off center cells The modulation depth was 50 per cent The plots have not been corrected for the phase shift introduced by the averaging procedure (see p 367) It can be seen that at the very lowest frequencies there is a phase lead, i e the on center response reaches its peak prior to the luminance maximum, the off center response prior to the luminance minimum (Correction for the error due to the averaging equipment would increase the amount by which the response leads at these low frequencies) As the stimulus frequency increases the phase lead turns into a steadily increasing phase lag the increase being more pronounced for the on than for the off center cells

Discussion

1 *Immobilization of the eye* We have found the dose rates of muscle relaxants commonly used in experiments on cat retinal ganglion cells far too small for our purposes The majority of our receptive fields were in the peripheral retina (see Fig 2) and therefore most likely had field centers whose diameters were 2 deg or more Wiesel 1960, Enroth Cugell and Robson 1966) Thus, as a rule our stimulus diameter less than 1 deg) was small compared to the diameter of the receptive field center Considering that the sensitivity falls off quite rapidly with distance from the central point of maximum sensitivity (Kuffler 1953, Rodieck and Stone 1965b Enroth-Cugell and Robson, 1966) one expects the steady state output of the cell in response to a restricted stimulus to be critically dependent upon the position of the stimulus within the receptive field center It is possible that other workers have had less need for longlasting perfect immobilization of the eye because (1) they have not been interested in quantitative aspects of the steady state response of the cell or (2) they have been studying the steady state behavior but used a stimulus much larger

than ours compared to the diameter of the receptive field center Ogawa *et al* (1966) studied steady state responses from cat retinal ganglion cells with receptive field centers smaller than ours (all their recordings were from area centralis) while maintaining muscle paralysis with 8 mg/kg/hr of gallamine triethiodide Yet their smallest stimulus was 1 4 deg Possibly the mechanical support of the eye employed by these authors was partially responsible for their findings that "there was usually no apparent change in the discharge characteristics of the ganglion cells over the long periods (more than 4 hrs) that were required to complete the detailed study of each unit" On the other hand, they also state 'under apparently steady state conditions both the spontaneous and the evoked discharges would occasionally show temporary alterations in the mean firing rate" Perhaps these alterations were caused by eye motions similar to those we believe responsible for the slow fluctuations in mean frequency of discharge that were so disturbing in our experiments

The gallamine triethiodide dose rates with which the majority of the results reported here were obtained are three to five times larger than those reported by others One may question if such large doses possibly have an effect upon the central nervous system in general and on retinal function in particular We have noted no retinal ganglion cell behavior that we suspect is related to the gallamine administration nor have we found any indication in the literature that such effects could be expected

2 *Response characteristics* In our study of retinal ganglion cell response characteristics during stimulation with sinusoidally modulated lights we focused our attention on four aspects of the stimulus-response relationship mean discharge frequency relative response amplitude, the waveform of the response and the phase shift, Among a total of 31 cells we found only two cells (no 16 and 6) which, at constant frequency of stimulation (8 cps), behaved sensibly linearly up to approximately 40 per cent modulation in the following 3 respects (1) the mean discharge frequency of the cell did not depend upon the modulation depth, (2) the response amplitude was reasonably proportional to the depth of the stimulus modulation, (3) the waveform of the response was almost sinusoidal However, the claim that these two cells (over a limited range of modulation depths, at the one mean luminance level that we used) in fact operated linearly requires their stimulus response phase-shift to be independent of modulation depth (to 40 per cent) For the on center cell (no 16) this was true, but for the off center cell it was not Among all the off-center cells this particular one (no 6) exhibited the least increase in phase shift at low modulations (see square symbols Fig 8B) Thus within the restricted conditions of our experiments, only these two cells appeared to behave reasonably linearly but it should be remembered that we did not use different mean luminances nor did we do any superposition experiments

The remaining 29 cells behaved somewhat differently In general terms if one of the 4 aspects of the output that we studied showed a relatively linear relationship to the stimulus, one or more other aspects did not For example, all the off center curves relating response amplitude to depth of modulation in Fig 5B are tolerably linear

up to high modulation depths Yet the mean discharge freque of all these cells (except no 6) depended upon the modulation depth and for some of the cells the waveform was grossly distorted even at the very lowest modulation depths

Our cell sample is not large, does not include many cells from the central retina and our experimental data are incomplete in some aspects In spite of these short comings our results contradict such generalizations as the retinal ganglion cell discharge can be modulated in an almost linear fashion by photic sine wave stimuli (Hughes and Maffei quoted from Maffei *et al* 1965) However Hughes and Maffei used diffuse retinal illumination and no details of their results are available Rackensperger *et al* (1965) stimulated the receptive field center and their very short preliminary report does not indicate linear retinal ganglion cell behavior The results presented here are in agreement with those of Enroth Cugell and Robson (1966) They concluded that photoreception, initial signal transmission and summation was linear for some cat retinal ganglion cells but they made no claim that the response of a ganglion cell is linearly related to the stimulus magnitude It is interesting to note that although we in general found retinal ganglion cell responses to be nonlinear we did not note any threshold type nonlinearities although some cells were tested at modulation depths as low as 0 25 per cent This agrees well with the findings of Enroth Cugell and Robson (1966) in experiments with moving sinusoidal grating patterns

In spite of variations between individual cells of on and off center type there was a trend to consistent differences between the two groups The mean discharge frequency at 0 per cent modulation was on an average higher for on center cells as must be expected with a stimulus located in the receptive field center (*Cf* Fig 3A and 3B) A saturation type nonlinearity was typical for on center cells but not for off center cells in the curves relating response amplitude to modulation depth at a constant frequency of stimulus There was a greater decrease in phase lag with increase in stimulus modulation depth as well as a more pronounced increase with rising stimulus frequency for the on center cells than for the off center cells Marked deviation of the waveform of the response from a sinusoid mostly took the form of rectification in the case of off center cells double peaks were also observed For on center cells the distortion was more often of the type seen in Fig 6 at the highest depths of modulation occasionally in conjuncation with clipping

The frequency characteristics of both on and off center *responses* (suprathreshold conditions) all had the same general shape a maximum at an intermediate frequency of stimulation falling off towards both lower and higher frequencies The modulation *sensitivity* curves (*i e* frequency characteristics at threshold) were all obtained with an auditory method Properly such curves should be objectively determined response samples should be recorded for each stimulus frequency at several depths of modulation From the averaged data the modulation depth which at each frequency results in a constant small response could then be found Such procedure is however too time consuming to be realistic Sensitivity curves auditory type are clea

ganglion cell involved but also an auditory threshold of the experimenter. Further it is not unlikely that the criterion chosen by the experimenter varies with stimulus frequency and with the average discharge frequency of the cell. On the other hand, for any individual cell such modulation sensitivity curves can be repeated quite satisfactorily after a period of training. All the modulation *sensitivity* curves that we determined with the auditory method were of the same general shape as the frequency characteristics of retinal ganglion cell *responses*. They were also of the same general type as deLange's (1957) subjectively determined human modulation *sensitivity* curves. From our experiments it would thus appear that there is an analogy between psychophysical results obtained in humans with sinusoidal stimuli at threshold and the frequency response characteristics of retinal ganglion cells in the cat, both at "threshold" and at suprathreshold level. This suggests that the frequency response characteristics of the human visual system are at least partly determined at the retinal level, provided we are justified in arguing from the cat to the human visual system.

Notes added in proof

[illegible]

amplitude-modulation curves to yield suprathreshold plots of modulation sensitivity versus stimulus frequency at two different constant response levels. These sensitivity curves had the same general shape as the modulation sensitivity curve at "threshold" (Fig. 9C) and the response amplitude curves (Fig. 9A).

[illegible]

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Acta physiol. scand. 1966. 68. 382—396

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Circulatory and Respiratory Adaptation during Prolonged Exercise in the Supine Position

By

L G Ekelund

Received 27 May 1966

Abstract

Ekelund, L-G *Circulatory and respiratory adaptation during prolonged exercise in the supine position* Acta physiol scand 1966 *68* 382—396

Circulatory and respiratory changes during prolonged non steady state exercise in the supine position were studied by the heart catheterization technique in six healthy young men of average physical fitness The work load was chosen so that they could perform supine exercise for one hour This load gave a mean heart rate of about 128 beats/min after 10 min and 142 beats/min after 50 min of work The circulatory changes during these experimental conditions were characterized [illegible] characterized by a slight increase in total ventilation, an unchanged alveolar ventilation and a slight increase in dead-space ventilation The small increase in total ventilation was due to a small increase in respiratory rate and tidal volume The overall ventilation perfusion relationship was unchanged Both circulatory and respiratory changes may be explained by a decreased tonus in the capacity vessels with a shift in the distribution of the blood

In an earlier study (Ekelund and Holmgren, 1964) the adaptation of the circulation and respiration was studied during non-steady-state exercise for one hour in the sitting position During these experimental conditions there was a continuous fall in the stroke volume and systemic arterial blood pressure, but the cardiac output was maintained constant by an increase in heart rate The respiratory response was characterized by an increased total ventilation, with a moderate increase in alveolar ventilation and a marked increase in dead-space ventilation The overall ventilation-perfusion ratio of the lungs showed a slight continuous increase The circulatory and respiratory changes might be explained by a gravitational shift in the blood volume due to a decrease in the tone of the capacity vessels If so, the possibility of maintaining a steady state should be better in the supine than in the sitting position, when the influence of gravitation should be diminished

A preliminary report was given at the Meeting of the Swedish Society for Clinical Physiology, Stockholm, Nov 1963

In a metabolic study on a similar material Carlson *et al* … however found a fairly marked increase in heart rate during exercise for 60 min … supine position The present investigation was therefore planned to study whether … stroke volume behaves in the same way in supine as in sitting position under simil… experiment… conditions and with equivalent material

The circulatory response was studied by the heart catheterization technique and the respiratory changes by means of arterial blood and expired air samples

Material

The subjects consisted of 6 healthy male volunteers aged 20—28 … in normal … 'ank Prior … ECG at

Methods

The … The … cardic … Holm…

Right heart catheterization was performed by the conventional technique from the left arm with a No 9 double-lumen catheter The right brachial artery was catheterized percutaneously *Blood pressures* were recorded with an electrical transducer and with the reference point at the mid thoracic level The *cardiac output* was measured by the direct Fick method Expired air was collected in Douglas bags and was analysed for oxygen and carbon dioxide by the Haldane technique The heart *rate* was determined from … The respiratory … saturation and … Pernow 1959) … P_{CO_2}-electrode a… two cases P_{CO_2} was determined by the Astrup technique, to which values 3 0 mm Hg was added (Eke lund and Holmgren 1964, Holmgren 1965) The *pH standard bicarbonate and base excess* were de termined according to Andersen (1963) Measurements of pH and P_{CO_2} were corrected for differ ences between body temperature and electrode temperature *Lactic acid concentration* was determined by the spectrophotometric method of Barker and Summerson

The *statistical calculations* were performed according to Snedecor (1959)

Procedure

Prior to the experiment a pilot test was performed to choose a load that the subjects could sustain for one hour in supine position Because of the difficulty of exercising in supine position these loads were somewhat lower than those used in the earlier study in the sitting position (Ekelund and Holmgren 1964) Expressed in per cent of their working capacity at pulse 170 (W_{170}) the subjects worked at a level of 59 7 (57—63) % in supine against 77 (67—85) % in the sitting position

The experiment wa… tion or quinidine wa… brachial arteries card… bonate were measured … in the same body position and continued for one hour at the load selected in the pilot test The pedal axis of the bicycle was located 20—24 cm above the table with a stroke radius of 18 cm Every 10th minute expired air was collected for 3 min while blood samples were withdrawn

TABLE I Anthropometric data in 6 healthy young men The symbols are the same as in Fig 1—4

Case no	Age, years	Height, cm	Weight, kg	Heart, vol, ml	THb, g	Hb, konc, g/100 ml	Blood, vol., l	W_{170} kpm/min sitting	W_{170} kpm/min supine
1	23	192	86	960	985	13 8	7 80	1,250	1,400
2	28	191	79	965	800	13 4	6 60	1,100	1 150
3	21	186	83	915	915	14 7	6 80	1,150	1,250
4	24	180	72	800	705	13 8	5 60	1,000	1,050
5	24	174	68	650	560	12 3	5 05	1,000	950
6	20	187	69	740	700	12 3	6 25	1,150	1,250

the brachial and pulmonary arteries for the determination of oxygen saturation tension and content, hemoglobin concentration, carbon dioxide tension pH standard bicarbonate, base excess and lactate concentration After 10 and 40—50 min of work I^{131}labelled albumin was injected for blood volume determination. Rectal temperatures ECG heart rate brachial arterial pulmonary arterial and right ventricular pressures were monitored continuously In 3 subjects the pulmonary arterial wedge pressure was measured after 10 and 60 min work The work test was continued for 60 min in 5 subjects No complications occurred before during or after the test The blood loss was of the order of 150 ml The total fluid losses (blood and sweat) were approximately compensated for by continuous saline administration. The room temperature was between 21 and 23 5 C with a relative humidity of about 40 %

Results

Anthropometric data are given in Table I

The total amount of hemoglobin (THb) was on an average 778 g (range 760—985) corresponding to 10 1 g per kg b w (range 9 8—11 6), which is about 10 per cent lower than earlier values reported from this laboratory (Holmgren *et al* 1960) This difference is due to a difference in absolute calibration of the carbon monoxide reference gas The mean value for the hemoglobin concentration was 13 4 per 100 ml blood (range 12 3—14 7), which is of the same magnitude as in the abovementioned material

The total blood volume determined by the alveolar carbon monoxide method was on an average 6 35 l (range 5 05—7 80) or 83 1 ml (range 74 3—90 7) per kg b w

The total blood volume, determined with I^{131}labelled albumin, was on an average 5 88 (range 5 0—7 0) l in 5 subjects The CO blood volume for the same individuals was on an average 6 06 l After 10 min work the blood volume had decreased to an average of 5 62 l, a decrease of 260 ml or 4 4 per cent of the initial value (not significant) After 50—60 min work the average blood volume was 5 54 l which is not significantly different from the value after 10 min work The corresponding plasma volumes were 3 64 l at rest, 3 40 l after 10 min work, and 3 38 l after 50—60 min work

The heart volume in the prone position averaged 838 ml (range 650—965)

The rate of work that could be performed at the heart rate of 170 beats per min (W_{170}) was on an average 1063 kpm/min (range 1000—1250) in the sitting position There was no significant difference between W_{170} in the supine and sitting position (average 1138 kpm/min in supine)

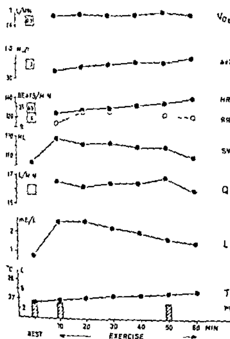


Fig 1

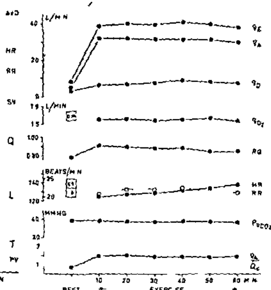


Fig 2

Fig 1 Hemodynamic data at rest, data in squares, and during exercise for one hour in six subjects V_{O_2} = oxygen uptake, l STPD/min AVD = arteriovenous oxygen difference ml/l HR = heart rate beats per min, RR = respiratory rate breaths per min SV = stroke volume ml Q = cardiac output, l/min, L = lactic acid in arterial blood, meq/l, T = rectal temperature C PV = plasma volume, l

Fig 2 Respiratory data at rest and during exercise in 6 subjects V_E = total ventilation l BTPS/min, V_A = alveolar ventilation, l BTPS/min V_D = deadspace ventilation, l BTPS/min V_{O_2} = oxygen uptake l STPD/min, RQ = respiratory quotient HR = heart rate beats/min RR = respiratory rate breaths/min, Pa_{CO_2} = arterial carbon-dioxide tension mm Hg V_A/Q_C = ventilation perfusion relationship

The anthropometric data indicate that this material did not differ significantly from the material used for the study in the sitting position Ekelund and Holmgren 1964) with the exception of the pulse rate in standing position which was on an average 88 7 beats/min in this material and 77 8 beats/min in the earlier with the same resting pulse rate

Circulatory response The data are presented in detail in Table II and Fig 1 and 2 *The oxygen uptake* (V_O) *at rest* in the supine position was 10 6 3 9 — 25 0) of that predicted (Harris and Benedict 1919) During exercise V_{O_2} after 10 min work had increased to an average of 1607 ml/min (range 1300—1950 correspond-ing to a mechanical efficiency of 25 6 per cent range 23 3 27 5 During the prolonged work there was an insignificant increase in V_{O_2} to an average of 1655 ml/min (range 1360 2000), corresponding to a mechanical efficiency of 24 8 per cent (range 22 9— 27 1) after 50 min work

TABLE II Data obtained at rest and during exercise with heart catheterization in 6 healthy young m

Case no	Work load kpm/min	Time min	Pulse rate, beats/min	$\dot V_{O_2}$, ml STPD/min	$\dot V_E$ l BTPS/min	O_2 cap, ml/100 ml	AVD, ml/l	Q, l/min	SV ml	Lactic acid meq/l
1	rest		68	360	10 4	19 4	48	7 4	108	0 58
	800	10	120	1,950	46 1	20 9	106	18 4	153	2 05
	800	20	122	1,930	47 0	21 1	109	17 8	146	1 60
	800	30	124	1,980	47.5	21 5	102	19 3	156	1 75
	800	40	134	2 000	50 0	21 5	102	19 6	146	1 45
	800	50	132	2 030	50.2	21 7	105	19 4	147	1 25
2	rest		61	340	9.5	18.3	43	7 8	128	0 80
	700	10	128	1,550	42 4	19.8	91	17 1	134	5 00
	700	20	130	1,580	43 3	20 0	99	16 0	123	6 45
	700	30	135	1,560	40 4	20 1	96	16 2	120	5 10
	700	40	132	1 490	40 9	20 1	102	14 6	111	4 37
	700	50	138	1,580	36 9	20 5	99	16 0	116	2 98
	700	60	136	1,590	36.2	20 7	103	15 5	114	2 20
3	rest		77	310	8 3	19 5	40	7 6	99	1 07
	750	10	133	1 710	37 6	20 9	92	18 6	139	2 45
	750	20	140	1 750	38 8	20 6	97	18 1	129	1 75
	750	30	142	1 740	38 8	20 7	105	16 6	117	1 57
	750	40	146	1 780	39 1	20 9	102	17 5	120	1 16
	750	50	147	1 820	40 5	21 3	104	17 6	120	1 03
	750	60	157	1,830	40 2	20 9	107	17 2	109	1 03
4	rest		62	250	6 4	18 8	40	6 2	100	0 58
	600	10	126	1 480	33 0	19 5	101	13 8	106	1 74
	600	20	130	1 470	34 4	20 3	107	12 8	99	1 42
	600	30	129	1,370	33 9	20 0	102	13 5	105	1 52
	600	40	136	1 420	39 9	20 1	106	13 4	98	1 59
	600	50	145	1 450	36 2	20.2	101	14 4	100	1 76
	600	60	155	1 410	36 1	20 3	105	13 6	86	0 68
5	rest		72	240	7 9	16 5	43	5 5	76	0 57
	600	10	130	1 300	32 9	17 4	90	14 5	111	2 03
	600	20	133	1 310	33 9	17 4	87	15 1	114	1 54
	600	30	135	1.290	33 9	17 6	86	14 9	111	1 20
	600	40	138	1 330	33 7	17 7	87	15 3	111	1 23
	600	50	144	1 360	34 3	17 7	95	14 7	102	0 96
	600	60	142	1 370	34 4	17 5	95	14 4	102	1 10
6	rest		70	290	9 1	17.3	37	7 9	113	1 04
	750	10	132	1 650	41.2	18 3	96	17.2	130	2 55
	750	20	136	1 650	43.2	18.3	94	17 6	130	3 00
	750	30	140	1 660	46 0	18.2	90	18 4	132	2 63
	750	40	138	1 630	43 2	17 9	89	18 4	133	2 50
	750	50	143	1 690	43 9	17 9	87	19 4	135	2 25
	750	60	144	1 730	45 3	18 0	91	19 1	132	2 37

…nm The symbols are the same as in Fig 1—4

Pressure mm Hg									Blood vol	Plasma vol
RV		PA			PCV	Br A				
S	D_e	S	D	M	M	S	D	M	l	l
35	–	24	15	20	-	130	77	91	–	–
45	–	37	22	30	–	153	70	103	–	–
–	–	32	19	26	–	150	71	103	–	
38	–	31	17	25	–	155	76	103	–	–
40	–	31	17	25	–	156	82	112	–	–
35	–	31	16	24	–	156	85	109	–	–
41	7	19	9	15	13	122	69	89	6 0	3 6
38	2	31	15	22	–	180	98	121	6 0	3 6
37	2	26	10	18	–	177	90	120		–
33	1	25	14	17	–	170	87	113		–
33	1	24	10	17	–	160	84	110	5 6	3 4
28	1	22	10	17	–	162	85	112	–	–
29	1	24	10	17	9	157	85	112	5 6	3 4
23	9	17	11	15	10	118	68	87	7 0	4 1
41	4	30	15	24	–	147	69	103		–
31	2	32	15	20	13	139	66	92	6 0	3 5
40	2	29	12	17	–	132	63	87		–
39	-1	27	11	17	–	133	65	88		–
38	1	27	11	18	–	125	62	87	6 0	3 4
40	2	27	13	19	13	117	60	82		–
47	7	20	10	15	10	123	67	87	5 2	3 2
38	1	29	16	22	17	140	73	94	5 3	3 1
–	–	24	10	16	–	130	66	82		–
35	1	23	10	17	–	120	59	80	–	
35	1	23	8	17	–	120	59	77	–	
35	1	25	10	17	–	123	60	81	5 1	3 1
36	2	26	12	16	8	115	56	78	–	
70	8	25	10	15	9	105	64	79	5 0	3 3
38	7	34	21	28	18	136	80	100	4 7	3 0
31	3	30	18	24	–	143	73	100	–	
17	3	32	18	24	–	132	68	93		–
35	2	29	17	22	–	133	68	93	4 7	3 0
37	2	33	17	22		132	68	89	4 7	3 0
37	1	32	14	21	11	125	66	87	–	–
19	6	15	8	12	9	119	70	85	6 2	4 0
46	5	36	18	24	–	[illegible]	69	96	–	–
41	3	30	15	23	–	–	–	–	6 1	3 8
45	3	30	14	21	–	139	67	90	–	–
4	3	26	13	19	–	137	63	93	6 2	3 9
45	3	29	13	21	–	144	65	91	–	–
45	5	34	15	23	12	141	64	93	6 3	4 0

*—M.SM? Acta physiol scand 1 » 68 3—4

TABLE III Respiratory data at rest and during exercise in 6 healthy young men The symbols are a

Case no	Work load kpm/min	Time min	Resp rate breaths/min	$\dot V_E$ l BTPS/min	V_T ml BTPS	$\dot V_{O_2}$ ml STPD/min	RQ
1	rest		16	10 4	650	360	0 78
	800	10	22	46 1	2 100	1 950	0 88
	800	20	24	47 0	1 960	1 930	0 91
	800	30	25	47 5	1 900	1 930	0 89
	800	40	24	50 0	2 080	2 000	0 90
	800	50	26	50 2	1 930	2 030	0 88
2	rest	–	12	9 5	790	340	0 81
	700	10	18	42 4	2 350	1 550	0 99
	700	20	15	43 3	2 880	1 580	0 98
	700	30	13	40 4	3 110	1 560	0 90
	700	40	13	40 9	3 150	1 490	0 97
	700	50	10	36 9	3 690	1 580	0 86
	700	60	12	36 2	3 020	1 590	0 84
3	rest		17	8 3	490	310	0 75
	750	10	18	37 6	2 090	1 710	0 88
	750	20	22	38 8	1 760	1 750	0 84
	750	30	18	38 8	2 160	1 740	0 87
	750	40	17	39 1	2 300	1 780	0 85
	750	50	17	40 5	2 380	1 820	0 83
	750	60	17	40 2	2 370	1 830	0 85
	rest		16	6 4	400	250	0 75
	600	10	15	33 0	2 200	1 480	0 91
	600	20	17	34 4	2 020	1 470	0 85
	600	30	19	33 9	1 790	1 370	0 88
	600	40	22	39 9	1 820	1 420	0 93
	600	50	19	30 2	1 900	1 450	0 82
	600	60	22	36 1	1 640	1 410	0 87
5	rest		19	7 9	420	240	0 80
	600	10	21	32 9	1 570	1 300	0 89
	600	20	20	33 9	1 700	1 310	0 91
	600	30	2	33 9	1 540	1 290	0 88
	600	40	22	33 7	1 530	1 330	0 87
	600	50	2	34 3	1 560	1 360	0 84
	600	60	2	34 4	1 570	1 370	0 84
6	rest		18	9 1	510	290	0 84
	750	10	23	41 2	1 790	1 650	0 94
	750	20	26	43 [illegible]	1 660	1 650	0 94
	750	30	27	46 0	1 700	1 660	0 96
	750	40	28	43 2	1 550	1 630	0 90
	750	50	28	43 9	1 570	1 690	0 92
	750	60	27	45 3	1 680	1 730	0 92

* *As cup-determ*inations with a corr n f 3 mm Hg

the same as in Fig 1—4

V_D, ml BTPS	V_D, l BTPS/min	Rectal temp °C	Arterial pH units	Arterial P_{CO_2}, mm Hg	Stand. bicarb meq/l	V_A l BTPS min	V_A/Q_C
221	4 2	36 6	7 43	39*	25	6 2	0 84
274	6 9	37 0	7 42	38*	23	39 2	2 13
253	7 1	37 1	7 42	38*	23	39 9	2 24
30?	8 6	37.2	7 43	39*	24	38 9	? 02
398	10 5	37 4	7 43	40*	24	39 5	2 02
423	12 0	37 5	7 43	41*	24	38 ?	1 97
230	3 2	36 6	7 44	38*	24	6 3	0 81
300	7 2	37 0	7 36	38*	20	35 2	2 06
364	6 1	37 2	7 38	36*	20	37 2	2 33
364	5 3	37 4	7 38	35*	20	35 1	2 17
495	6 9	37 5	7 40	35*	21	34 0	2 33
476	5 2	37 6	7 41	37*	22	31 7	1 98
443	5 8	37 7	7 42	38*	23	30 4	1 96
160	3 4	36 9	7 39	41	23	4 9	0 64
231	4 9	37 1	7 38	40	22	32 7	1 76
313	7 8	37 3	7 39	41	24	31 0	1 71
391	7 7	37 4	7 39	42	23	31 1	1 87
443	8 2	37 5	7 40	43	23	30 9	1 77
508	9 3	37 6	7 41	42	24	31 2	1 77
338	6 4	37 7	7 42	40	23	33 8	1 96
127	2 7	36 7	7 41	44	25	3 7	0 59
312	5 2	37 1	7 42	42	24	27 8	2 01
324	6 2	37 3	7 43	39	24	28 2	2 20
388	8 1	37 5	7 43	41	25	25 8	1 91
377	9 1	37 7	7 46	37	25	30 8	2 29
303	6 5	37 8	7 46	39	25	29 7	2 06
354	8 7	37 9	7 45	39	?5	27 4	2 02
151	3 6	36 5	?39	39	21	4 3	0 78
273	6 6	36 9	38	39	?1	26 3	1 81
333	7 5	37 1	7 40	39	22	26 4	1 75
268	6 8	3 2	41	37	22	27 1	1 82
327	8 1	3 4	7 40	39	?2	25 6	1 67
334	8 2	3 5	41	38	22	26 1	1 77
304	9 6	37 6	40	3	21	24 8	1 72
142	3 3	36 9	7 41	37	22	5 8	0 74
282	7 4	37 1	7 34	40	20	33 8	1 96
392	9 3	37 3	7 36	42	21	31 9	1 81
420	12 4	37 6	7 36	41	20	33 6	1 82
393	12 1	37 7	7 36	41	21	31 1	1 69
321	10 1	37.8	7 37	40	21	33 8	1 74
363	10 8	37 9	7 37	40	20	34 5	1 80

The heart rate at rest was on an average 68.3 beats per minute (range 61—77). During exercise it rose to an average of 128.2 beats per min (range 120—133) after 10 min work. Continued work increased the heart rate in five-sixths of the subjects to an average of 141.5 beats per min (range 132—147) after 50 min work. In subjects who exercised for 60 min there was a further increase of, on an average, 3.4 beats per min (range 142—157).

The oxygen saturation of arterial blood was normal (mean 96.9 per cent) at rest and remained unchanged during the whole work period (97.3 and 97.2 per cent mean value after 10 and 60 min work).

The cardiac output at rest was on an average 7.1 litres *per minute (range 5.5—7.9)*. During exercise cardiac output increased after 10 min to an average of 16.6 l/min (range 13.8—18.6). During the continued work the cardiac output showed no significant change, the mean value after 50 min being 16.9 l/min (range 14.4—19.6). All values at rest and during work fell within the normal limits predicted from the normal regression of cardiac output to oxygen uptake published from this laboratory (Bevegård *et al.* 1960).

The stroke volume at rest in supine position was on an average 104.0 ml (range 76—128). After 10 min exercise the stroke volume had increased in all subjects to an average of 128.8 ml (range 106—153), an increase of 23.8 per cent above the resting volume. In all subjects the stroke volume decreased continuously during the work period to an average of 120.0 ml (range 100—146) after 50 min, an in-
change ($0.1 > P > 0.05$). In the 5 subjects who worked for 60 min
was a further decrease of 6 ml during the last 10 min ($P < 0.05$). The volume
however, still larger than at rest. The 10-min values fell within the normal
range of variation for the relationship between the stroke volume during exercise in supine position and total hemoglobin, heart volume and W_{1-6} (Holmgren, Jonsson and Sjöstrand 1960).

Intracardiac and intravascular pressures

The pulmonary wedge pressure (PCV) was recorded at rest in five, after 10 min in two and after 50 min in 5 subjects. At rest the PCV pressure was on an average 10.2 mm Hg (range 9—13) and after 60 min work 10.6 mm Hg (range 8—13) ($n = 5$). In two subjects the averages were 9.5 mm Hg, 17.5 and 9.0 mm Hg at rest, after 10 and 60 min work respectively.

The systolic right ventricular pressure was at rest, on an average 22.8 mm Hg (range 19—27) and rose after 10 min exercise to an average of 41.0 mm Hg (range 38—46). There was an insignificant decrease to a mean of 36.3 mm Hg (range 28—45) after 50 min work (mean change — 4.7 mm Hg). *The end-diastolic pressure in the right ventricle* ($n = 5$) at rest was on an average 7.4 mm Hg and fell significantly ($P < 0.05$) after 10 min work to an average of 3.8 mm Hg and after 60 min work to 2.2 mm Hg, a further decrease of 1.6 mm Hg but insignificant.

The systolic pulmonary arterial pressure was at rest, on an average 20.0 mm Hg (range 15—25), rose after 10 min work to 32.8 mm Hg (range 29—37), and de-

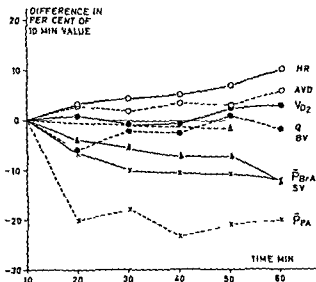


Fig 3 Hemodynamic responses during long term exercise in supine position in six subjects $\bar{P}_{BR_A}$ = brachial arterial mean pressure, $\bar{P}_{PA}$ = pulmonary arterial mean pressure, BV = total blood volume The other symbols are the same as in Fig 1 Data are presented as a percentage difference from the values obtained after 10 min work

creased after 50 min work to 27 8 mm Hg (range 22—33 mm Hg, mean change — 5 0 mm Hg) *The mean pressure in the pulmonary artery* was, at rest, on an average 15 3 mm Hg (range 12—20), 25 0 mm Hg (range 22—30) after 10 min work and 19 8 mm Hg (range 17—25) after 50 min work, a decrease of 5 2 mm Hg between 10 and 50 min

The decrease in mean pressure is highly significant ($P < 0\ 001$) and is mainly a result of a decrease in the diastolic pressure

The systolic brachial pressure was on an average 119 5 mm Hg (range 105—130) at rest, 150 2 mm Hg (range 136—180) after 10 min work and 140 3 mm Hg (range 123—156) after 50 min work (mean change — 9 8 mm Hg) with a further decrease of 6 mm Hg for the 5 subjects who worked for 60 min The corresponding figures for diastolic pressure were 69 2 mm Hg (range 64—77), 76 5 mm Hg (range 69—98) after 10 min work and 71 3 mm Hg (range 60—88) after 50 min work The average mean pressure was at rest 86 3 mm Hg (range 85—91), 102 8 mm Hg (range 94—121) after 10 min work and 95 8 mm Hg (range 81—115) after 50 min work (mean change — 7 0 mm Hg), with a further decrease of 1 6 mm Hg for the 5 subjects who worked for 60 min The decrease from 10—60 min is significant ($P < 0\ 01$) The pulse pressure increased from 52 0 mm Hg at rest to 73 7 mm Hg after 10 min work and then decreased to 71 0 mm Hg after 50 min work The decrease in the systemic arterial mean pressure with a constant cardiac output indicates a decrease in the systemic vascular resistance

Respiratory response

The results of the respiratory studies are presented in detail in Table III and Fig 2 and 4

The minute ventilation ($\dot{V}_E$) increased from an average value of 8 6 l BTPS (range 6 4—10 4)

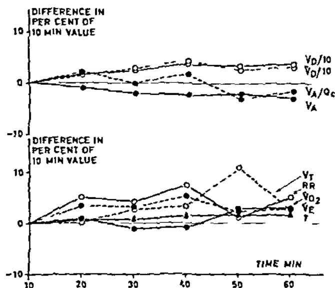


Fig 4 Respiratory responses during long term exercise in supine position V_D = physiological dead space ml BTPS/min V_T = tidal volume ml BTPS/min The other symbols are the same as in Fig 2 Data are given in percentage difference from 10 min value

It increased slightly to an average of 40 3 (range 34 3—50 2) after 50 min work This increase was caused by a combined increase of tidal volume (V_T) from 2 02 l to 2 17 l between 10 and 50 min and of *respiratory rate* from 19 5 to 23 3 breaths/min

Alveolar ventilation (V_A) increased from an average value of 5 2 l BTPS/min (range 3 7—6 3) at rest to an average of 32 5 l BTPS/min (range 26 3—39 2) after 0 min work V_A remained unchanged during the continued work, mean value of 31 8 l BTPS/min (range 26 1—38 2) after 50 min work, the slight increase in V_E being caused by an increase in the dead-space ventilation (V_D) due to an increase both of respiratory rate and of V_O V_D increased from an average of 6 4 l BTPS min range 4 9—7 4 after 10 min to 8 6 l BTPS/min (range 5 2—10 5) after 50 min work ($P < 0.05$)

The respiratory quotient, *RQ*, at rest averaged 0 79 (range 0 75—0 84) After 10 min work RQ had risen to 0 92 (range 0 88—0 99) It decreased slightly to an average of 0 86 range 0 82—0 92) after 50 min work ($P < 0.05$)

The arterial carbon dioxide tension (Pa_{CO_2}) at rest averaged 39 7 mm Hg (range 37—44) During work Pa_{CO_2} remained constant, on an average 39 5 and 39 3 mm Hg after 10 and 50 min work

The pH of arterial blood was on an average 7 41 (range 7 39—7 44) at rest After 10 min work there was a slight decrease to an average of 7 38 (range 7 34—7 42 and then a slight increase to an average of 7 42 range 7 37—7 46) after 50 min work

The standard bicarbonate at rest averaged 23 3 meq l (range 21—25) It decreased slightly to an average of 21 7 meq l range 20—24 after 10 min work After 50 min work the value had returned to about the same as at rest, on an average 23 0 meq/l (range 21—25)

Base excess at rest averaged — 0 85 meq l (range — 4 5— + 2 2) During work it decreased to an average of — 2 15 meq l range — 5 0— + 0 5) after 10 min and to — 1 45 meq/l (range — 4 5— — 1 9) after 50 min

The lactic acid concentration in arterial blood at rest averaged 0 77 meq/l During exercise it rose to an average value of 2 60 meq/l after 10 min work and then decreased to 1 71 meq/l ($P < 0.05$) after 50 min work

The overall ventilation perfusion relationship of the lungs (V_A/QC)

The V_A/QC at rest averaged 0 73 (range 0 59—0 84) After 10 minutes' work the V_A/QC had risen to 1 96 (range 1 8—2 1) During the continued work it showed no significant change, the value after 50 min work being on an average 1 88 (range 1 7—2 1)

Rectal temperature

The rectal temperature at rest averaged 36 7° C (range 36 5—36 9) During the work period there was a continuous increase to an average of 37 6 C (range 37 5—37 8) after 50 min work, with an further increase of 0 1° C for the 5 subjects who worked for 60 min

Discussion

The subjects used in the present investigation consisted of healthy young men (mean age 23 3 years) of ordinary physical fitness They were slightly taller and had a higher pulse rate in standing position than the subjects used in an earlier study in the sitting position (Ekelund and Holmgren 1964) The other anthropometric data studied indicated no further differences Because of the difficulty of exercising in the supine position the same level of working intensity as in the previous study could not be obtained without previous training of the subjects A work load was therefore chosen which the subjects could sustain for one hour A higher working intensity during a shorter time would perhaps show larger changes per unit time, but the accuracy of estimation of, for example the cardiac output by the Fick method might then be reduced

The mean pressure in the pulmonary artery decreased by 5 mm Hg (20 per cent) between 10 and 20 min work, and then remained unchanged In the two cases when also the wedge pressures were obtained there were more marked changes in the pulmonary arterial mean pressure than in the pressure drop over the pulmonary vascular bed A change of the arterial mean pressure in the pulmonary artery could be a result of a change of the pulmonary vascular resistance a change of capacity (expressed as change of volume per change of pressure, dV/dP) of the pulmonary vascular bed an altered distribution of the blood volume between the systemic and lesser circulation, or a change in the intrathoracic mean pressure as a result of an altered thoracic mid position From the observation of the wedge pressures a change of resistance does not seem to be the major factor The absolute intrathoracic pressure level is not known in the present study But if it is unchanged two main factors remain, a changed capacity of the pulmonary vessels and changed distribution of the blood volume

The pressure in the brachial artery showed a different pattern of resp

a continuous decrease which was a result of a corresponding decrease of the resistance in the systemic circulation

The size of the stroke volume is dependent on the dimensions of the heart and on the emptying and filling of the ventricles The filling of the ventricles is dependent on the available energy (kinetic and potential) and on the time for filling, and also on the mechanical properties of the myocardium The increase of the stroke volume from rest to 10 min work (+ 23 8 per cent) is due to a change of filling energy already at rest as a result of elevation of the legs and, at least in part, therefore, should be regarded as a change of 'base line" (Frick and Somer 1964) At the start of exercise there is also the positive effect of the muscular pump on the venous vessels in the legs The decrease in stroke volume during the continued work is the result of a combination of several of the above-mentioned factors *With the compensatory increase in heart rate the result will be an unchanged cardiac* output, which is fundamental for the maintenance of the optimal relation between cardiac output and oxygen consumption during work

A decrease in blood volume on the filling side of the heart could not be a result of a decrease in the total blood volume but must be due to an altered distribution of blood within the capacity vessels, as suggested by the change in the regional perfusion indicated by the increase in dead space ventilation

With the decrease of arterial mean pressure there will also be some cronotropic influence from the baroreceptor system with an acceleration of the heart rate, ing in a shorter filling period Influences from the baroreceptor system or the central nervous system may also have a negative inotropic effect on the heart muscle, with a change in the myocardial function Therefore for a certain level of work, there will be an optimal combination of filling energy and filling time The change in one of these components will change the steady state to another level of optimal combination

At the end of long continuous work there may be a change in cardiac metabolism with a greater part of the energy taken from an increased uptake of glucose break down of glycogen and an increased production of lactate Such a metabolic change may result, among other things, from an increased sympathetic influence on the heart

The minute ventilation increased slightly during the continued work but the alveolar ventilation was unchanged, the increase being therefore due to increased dead space ventilation The overall ventilation perfusion relationship of the lungs was unchanged during the continued work, but with the increase in dead space there might be a small change in the regional ventilation perfusion relationship The altered total distribution of the blood which is of significance for filling of the heart, might affect the regional distribution to different parts of the lung less in the supine than in the sitting position, which would result in much smaller changes in dead space ventilation The baroreceptor system especially in the pulmonary vessels, could be responsible for some of the changes in ventilation during the prolonged exercise, i e increase of respiratory rate (Kinnison *et al* 1965)

Comparison with the study in sitting position (Ekelund and Holmgren 1964). The studies differed in the experimental conditions, with a lower absolute and relative work level in the supine position. The relative intensity of exercise can be expressed by the pulse rate after 10 min and indicates a load in relation to the maximal capacity of the individual in a short exercise test. The relative level of work was lower supine than in sitting position with a 10 minute heart rate of 128 beats/min and 148 beats/min, respectively. The absolute load was 700 kpm/min in supine and 925 kpm/min in sitting position. The difference in circulatory reaction could therefore be due both to different postures and/or different relative working intensities. In spite of the better possibilities of filling of the heart in the supine position with the legs elevated, there was a decrease in stroke volume which, however, after the same work period (50 min), was less in supine than in sitting position. There was also a fall in the systemic pressure and a pronounced fall in the pressure of the pulmonary circulation. The decrease of stroke volume, in spite of the better possibility of filling of the left ventricle, seems to be a result of a change in tonus of the capacity vessels with an altered distribution of blood. There may also be a change in the contractility with impaired systolic emptying of the heart as a result of a decreased sympathetic influence or of a less powerful response to the same sympathetic influence. In short time exercise Bevegård, Holmgren and Jonsson (1962) have shown that there are great differences in the circulatory adaptation during work in sitting and supine position. To evaluate how large the influence of posture is on the adaptation during prolonged exercise, one has to perform a study in sitting position at about the same relative working intensity as in the supine exercise.

This study was supported by grants from Svenska Idrottens Vetenskapliga Forskningsråd and from Riksidrottsförbundets poliklinikkommitté.

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Nervous Regulation of Blood Flow in the Subcutaneous Adipose Tissue in Dogs

By

S H NGAI[1], SUNE ROSELL and LARS R WALLENBERG

Received 31 May 1966

Abstract

NGAI, S H, S ROSELL and L R WALLENBERG *Nervous regulation of bl d flow in the subcutaneous adipose tissue in dogs* Acta physiol scand 1966 *68* 397 403

The ... mam ... blooc ... ml ...

was also related to the stimulus frequency Propranolol a β receptor blocking agent abolished the vasodilator response Reflex adjustment of the peripheral vasomotor tonus upon carotid artery occlusion and activation of the sympathetic cholinergic vasodilator nerves in the hypothalamus did not appreciably change the vascular resistance in the adipose tissue

Quantitative measurements of blood flow in the adipose tissue are lacking probably due to the fact that this tissue is diffusely located between other tissues The juxta position of adipose tissue and skin may make it difficult to determine its metabolism and blood circulation *in vivo* Recently we have succeeded in isolating canine subcutaneous adipose tissue with an intact blood and nerve supply (Oro, Rosell and Wallenberg 1965) This paper will report investigations on the nervous control of blood flow in canine adipose tissue Studies on the release of free fatty acids (FFA) from the adipose tissue with the same type of preparation are being reported elsewhere Rosell 1966)

Methods

F... ...ghing 8 and 14 kg were used Pentobarbitone sodium was used as anes- ... or peripheral ... nts concerned ... als were pre

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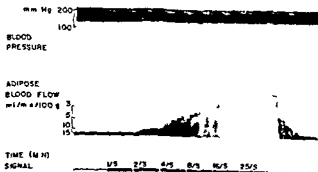


Fig. 1. Blood flow in subcutaneous adipose tissue in dog. Effects of electrical stimulation of the nerve to the adipose tissue. *Stimulus parameters: 8 V, 5 msec, frequency as indicated.*

medicated with morphine sulfate (1.0—1.5 mg/kg s.c.) and subsequently anesthetized with α-chloralose (100 mg/kg i.v.). The trachea was cannulated and artificial respiration provided with a Palmer Ideal pump when indicated. Heparin was given to prevent clotting.

... subcutaneous adipose tissue preparation is published elsewhere ... and directing the blood through a silicone-oil filled drop ... blood vessels were ligated. In 5 expts. muscle blood flow was also ... this muscle. In these experiments ... was placed in the femoral artery ... the artery supplying the muscle ... artery to the adipose tissue. The ...

end. The stimuli consisting of square pulses ... (5—8 V) and varying frequency were delivered from a Grass S 4 stimulator ... hypothalamic vasodilator outflow unipolar electrodes (0.5 mm ... ted into the brain through small burr ... the Horsley—Clarke technique. The ... 70—80 imp/sec with varied intensity.

Results

Resting blood flow and effects of nerve stimulation

In each experiment the resting flow in denervated adipose tissue was determined prior to any stimulation. The mean value of blood flow from 17 expts. was 8.5 ml/min · 100 g adipose tissue and varied between 3 and 19 ml/min · 100 g provided the arterial blood pressure was kept within its normal range. Stimulation of the appropriate sympathetic chain, or the nerve supplying the adipose tissue, decreased the blood flow, *i.e.* vasoconstriction occurred, the magnitude of which was frequency dependent. Fig. 1 shows the vasoconstrictor responses to electrical stimulation of the mixed nerve to the adipose tissue. Stimulation with 1/sec did not induce any significant change in the blood flow while an appreciable vasoconstriction occurred

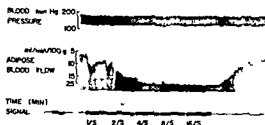


Fig 2 ... ergotamine was given i a prior to the recording Stimulus parameters 8 V, 5 msec frequency as indicated

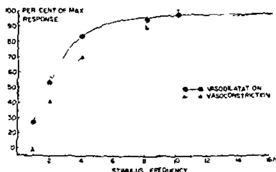


Fig 3 Stimulus response curves Electrical stimulation of the nerve to the subcutaneous adipose tissue in dog The vasomotor reactions are expressed in per cent of the maximal response obtained in each individual experiment ▲——▲ Vasoconstrictor responses (mean of 9 expts) The sequence of stimuli was 1, 2 4, 8 and 16/sec for 1 min at each frequency ●——● Vasodilatation responses (mean of 5 expts) 100 µg dihydroergotamine was administered i a prior to the stimulus series The sequence of the stimuli was the same as for the vasoconstrictor responses The vertical bars represent ± the standard error of the mean

... higher frequencies with a maximal effect being obtained with a stimulus frequency of about 16/sec Upon cessation of the stimulation the blood flow promptly returned to control values or, in most experiments, increased to values larger than control This secondary vasodilatation, if it occurred, was long lasting The vasoconstrictor responses to nerve stimulation are summarized in Fig 3 It is seen that 1/sec did not produce any appreciable change in blood flow while stimulation with 2/sec induced approximately 40 per cent of the maximal vasoconstriction obtainable The vasoconstricting effect of nerve stimulation was abolished by the i a injection of α receptor blocking compounds such as dihydroergotamine (25 100 µg) and phentolamine (100 µg)

Following α receptor blockade nerve stimulation invariably increased the blood flow Fig 2 illustrates one of these experiments in which dihydroergotamine was administered i a prior to the recording Stimulation with 1/sec significantly increased the blood flow above the control value with nearly a 4 fold increase occurring with stimuli of 4—16/sec The relationship between the vasodilatation and the stimulus frequency is illustrated in Fig 3 Although nerve stimulation als

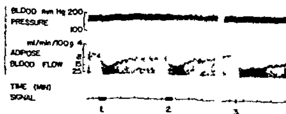


Fig 4 Blood flow in subcutaneous adipose tissue in dog 100 μg dihydroergotamine was given i a prior to the recording At 1 and 2, electrical stimulation of the nerve to the tissue with 3/sec and 5/sec respectively At 3 i a injection of 0 1 μg acetylcholine

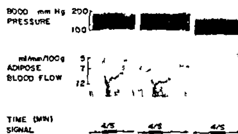


Fig 5 Blood flow in subcutaneous adipose tissue in dog Electrical stimulation of the nerve to the adipose tissue Dihydroergotamine 100 μg was administered prior to the recording Between the first and the second stimulations 100 μg atropine injected i a Between the second and the third stimulations 125 μg propranolol injected i a Stimulus parameters 5 V 10 msec 4/sec

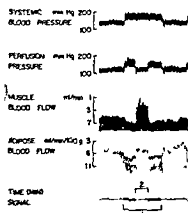


Fig 6 Blood flow in skeletal muscle and subcutaneous adipose tissue in dog Bilateral vagotomy 1 Bilateral carotid occlusion 2 Perfusion pressure adjusted to the pre-occlusion level during carotid occlusion

produced a vasodilatation after α-receptor blockade, the magnitude of change varied considerably from animal to animal In order to relate the effect of nerve stimulation to the maximal blood flow capacity of the vascular bed in the adipose tissue large doses of acetylcholine were injected i a to relax all smooth muscles in the vascular bed The increase in blood flow produced by acetylcholine was then compared with the maximal response to nerve stimulation Fig 4 shows that nerve stimulation increased the blood flow to approximately the same degree as the i a injection of acetylcholine with maximal blood flow value of about 25 ml/min · 100 g adipose tissue

The vasodilator response to nerve stimulation following α-receptor blockade was abolished by the administration of propranolol, a β receptor blocking drug Atropine had no effect in this respect (Fig 5)

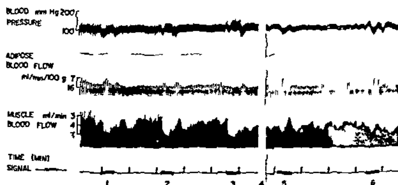


Fig 7 Blood flow in skeletal muscle and subcutaneous adipose tissue in dog Electrical stimulation of the sympathetic cholinergic vasodilator outflow in the hypothalamus Stimulus parameters 5 msec 80 sec

1 stimulation 1 V		4 0 5 mg atropine i a	
2 » 1 5 V		5 stimulation 1 5 V	
3 » 2 0 V		6 » 2 0 V	

Effects of reflex activation of vasomotor nerves

Occlusion of the common carotids was performed in 4 vagotomized animals in order to study the effect of reflex activation of the sympathetic outflow to the adipose tissue Blood flow in skeletal muscle was also measured in these experiments for comparative purposes Fig 6 illustrates one experiment in which carotid occlusion increased the mean arterial pressure from 125 to 170 mm Hg Blood flow increased in both the adipose tissue and the muscle However vasoconstriction in the muscles became evident when the perfusion pressure was adjusted to the pre occlusion level by tightening the screw clamp around the aorta The adipose tissue blood flow was not significantly different from the pre occlusion value and a vasoconstriction of minor degree was observed in only one experiment In 4 animals the mean reduction in the blood flow in the muscles with constant perfusion pressure was about 50 per cent during carotid occlusion

Effect of activation of the cholinergic vasodilator nerves

The cholinergic vasodilator outflow in the anterior hypothalamus was stimulated electrically in 3 expts in order to further study the vasomotor regulation of the vascular bed in adipose tissue One of these experiments is illustrated in F Stimulation resulted in an increased blood flow in the skeletal muscl adipose tissue blood flow remained practically unaffected during

stimulation However, a slight blood flow decrease may be noticed which is possibly due to changes in the arterial blood pressure The cholinergic nature of the vasodilator response in the muscle was indicated by the blocking action of atropine

Discussion

The present study indicates that the subcutaneous adipose tissue in dogs has a resting blood flow which is of the same order of magnitude as found under similar experimental conditions in the skeletal muscle Histological studies have shown a rich capillary network around fat cells Gersh and Still (1945) found in freeze dried rat adipose tissue that all fat cells were in contact with at least one capillary Later, Held and Rutishauser (1960) produced circulatory stasis to reveal vessels in the adipose tissue and observed an abundant vascular network Thus, the relatively high resting flow in the adipose tissue, as found in the present experiments is consonant with histological findings Larsen, Lassen and Quaade (1966), using local clearance of Xe^{133} found an average blood flow of 2 6 ml/min 100 g in the subcutaneous adipose tissue in man which is comparable to the blood flow in resting skeletal muscles in human subjects (see Shepherd 1963) Thus, in both dogs and man the resting blood flow seems to be of the same order of magnitude in the skeletal muscle as in the subcutaneous adipose tissue in the respective species In the present experiments stimulation of the sympathetic outflow or the nerve supplying the adipose tissue produced vasoconstrictor effects which were abolished by sympatholytic agents with α receptor blocking properties These findings indicate that vasoconstrictor nerves are sympathetic in origin and adrenergic in nature The data also show that the relationship between the stimulus frequency and the vasoconstrictor response is similar to that found in other sympathetically innervated tissues such as the nictitating membrane (Rosenblueth 1950) and the blood vessels of the skeletal muscle and the skin (Folkow 1952, Celander and Folkow 1953)

Nerve stimulation also elicited a vasodilator response provided that the vasoconstriction was blocked by α receptor blocking substances Even though sympathetic cholinergic vasodilator fibers are known to supply vascular beds in the skeletal muscle, and possibly the heart (Uvnas 1960) the adipose tissue is apparently not innervated by this type of fiber since activation of the sympathetic cholinergic vasodilator outflow did not produce any vasodilator response (Fig 7) Moreover, the vasodilator response induced by nerve stimulation was not blocked by atropine, but was abolished by propranolol a β receptor blocking agent (Fig 5) This finding indicates that although the vasodilator response is adrenergic it may be considered as a β receptor effect In this respect the vasodilator response is similar to the release of free fatty acids from the subcutaneous adipose tissue following sympathetic nerve stimulation (Fredholm and Rosell 1966 b) Whether vasoconstriction and vasodilatation are due to stimulation of the same or separate adrenergic nerves remains unanswered and requires further investigation (see Discussion, Rosell 1966)

A comparison of the increased blood flow resulting from either nerve stimulation or i a injection of acetylcholine reveals that nerve activity can produce a maximal

vasodilatation in the adipose tissue This indicates that the vasodilatation occurs in the resistance section of the vascular bed, *i e* arterioles A detailed study of the effects of nerve stimulation on consecutive sections of the vascular bed of subcutaneous adipose tissue will be published elsewhere (Öberg and Rosell 1966)

The vascular bed in the subcutaneous adipose tissue does not seem to participate to a significant extent in the reflex adjustments of the peripheral resistance elicited via the carotid baroceptors This is evident from the finding that occlusion of the carotid artery did not appreciably increase the vascular resistance in adipose tissue In this respect the vasomotor reaction in the subcutaneous adipose tissue seems to be similar to that found in the kidney (Lofving 1961)

This investigation has been supported by the Swedish Medical Research Council (Project no 14X 731 01), Stiftelsen Therese och Johan Anderssons Minne, and by the National Institutes of Health, Bethesda Md U S A (Ti HE 5614 04)

Propranolol (Inderal[R]) has been generously supplied by AB Scanmeda, Goteborg Sweden

We wish to thank Miss Mona Engqvist for skilful technical assistance

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Acta physiol. scand. 1966 68. 404—418

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Analysis of Odour Similarities from Electrophysiological Data

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Received 15 June 1966

Abstract

DOVING, K B *Analysis of odour similarities from electrophysiological data* Acta physiol scand 1966 *68* 404 418

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probability values were calculated for all pairs in a given series The statistical values were regard- to represent measurements of relatedness in olfactory stimulative properties between the tested [illegible]ances For each series the data were analysed with a nonmetric multidimensional scaling [illegible]d to give spatial representations of the relatedness between the odours The relation of the [illegible]ults to psychophysical findings and the classification of odours according to the stereochemical [illegible]ory are discussed

Increased knowledge about the organization of the atoms in odorous molecules has focused attention on stereochemical properties as important parameters in determining the quality of the odours The similarity in odour quality between substances of similar molecular size and shape has frequently been reported (Moncrieff 1951, Stoll 1957, Ruzicka 1957 Beets 1957 for review see Beets 1964) According to a specific stereochemical odour theory recently proposed by Amoore (1962) there are seven primary groups of odours Five of these groups camphoraceous ethereal, floral, minty and musky are based upon the steric resemblance of the substances within each of the groups The classification is made from common semantic descriptions of odours Although these descriptions as such may give valuable qualitative information they do not provide quantitative measurement of relatedness between the odours It is possible however by various available methods to obtain quantitative information about the relatedness between different odours (see *Discussion*) The psychological studies used to test the stereochemical theory of olfaction have provided evidence for a significant correlation between similarity of odour' and

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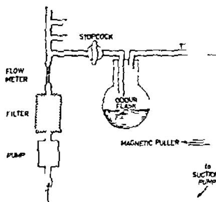


Fig 1 Schematicial drawing of the stimulating apparatus (see text)

'similarity of shape' (Amoore and Venstrom 1966, Amoore 1966 The
is an extension of previous electrophysiological investigations Dovin,
1966) and was undertaken with the intention to obtain data on the
between the stimulative properties of some odorous substances selected fro
roups of Amoore (1962) A statistical method of processing the electrophysio
ata is used and the results are compared with those obtained by psychophysical
methods In addition the statistical data are analyzed to give spatial representa
ion of the relatedness of the different odours

Methods

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TABLE I Substances used in the present experiments The impurities are given in per cent of the total amount of substance present

Substance	Structural formula	Source	Impurities (per cent)
Camphoraceous			
1 p-Dichlorobenzene	Cl— (ring) —Cl	Hopkin & Williams England	0 6
2 d, l-Camphor	(ring) O	Hopkin & Williams England	0 2
3 1, 8-Cineole	(ring) O	E Merck, Germany	0 2
4 2-Methyl 2-butanol	CH_3 CH_2 COH CH_3 CH_3	Hopkin & Williams England	< 0 1
5 3-Methyl 2-butanone	CH_3 CH CO CH_3 CH_3	Mallinckrodt USA	1 1
6 2 Methyl 2 propanol	CH_3 COH CH_3 CH_3	Hopkin & Williams England	0 1
Ethereal			
7 1, 2 Dichloroethane	ClH_2C CH_2Cl	Hopkin & Williams England	0 2
8 Carbon tetrachloride	C Cl_4	E Merck Germany	0 1
9 Furane	(ring) O	Hopkin & Williams England	—
10 1-Propanol	CH_3 CH_2CH_2OH	KEBO, Sweden	2 3
11 Pyrrole	(ring) H N		< 0 1

Substance	Structural formula	So	
1? Trichloroethene	ClHC=C Cl_2	E \ (	
Floral			
13 Acetophenone	O	Hopkin & England	0 8
14 Anisole	O	Hopkin & Willia England	
15 Benzophenone	O C	Hopkin & Williams England	0 1
16 Geraniol	OH	British Drug House	10 0
17 Salicylaldehyde	CHO OH	Hopkin & Williams England	0 1
M nty			
18 Cyclopentanone	O	Hopkin & Williams England	0 1
19 Cycloheptanone	O	Fluka, Switzerland	2 5
20 Cyclooctanone	O	Koch Light Lab England	2 1

Substance	Structural formula	Source	Impurities (per cent)
21 Diethylsulphate	$(CH_3CH_2)_2SO_4$	Hopkin & Williams England	1 3
22 l-Menthol	OH	Roure Bertrand Dupont, France	pure
23 l-Menthone	O	Roure Bertrand Dupont, France	pure
24 neo-Menthol*		Roure Bertrand Dupont, France	pure
25 neo-iso-Menthol*		Roure-Bertrand Dupont, France	pure
Musky			
26 5α-Androstan-3β-ol	HO	Koch Light Lab England	10 0
27 Celestolide* (1, 1-dimethyl-6 *tert* butyl-4 actyl indan	O	International Flavors & Fragrances The Netherlands	pure
28 Musk ketone (2,6-dimethyl 4 *tert* butyl 3 5-dinitro-acetophenone)	O_2N NO_2 O	International Flavors & Fragrances The Netherlands	pure
29 Musk lactone (15 hydroxypenta-decanoic acid lactone)	CH_2 O $(CH_2)_{13}$ C O	International Flavors & Fragrances The Netherlands	pure

Substance	Structural formula	Source	Impurities (per cent)
30 Musk I* (2-oxa 5,5,8,8-tetramethyl-1,2,3,4,5,6,7,8-octahydro-anthracene)		International Flavors & Fragrances The Netherlands	pure
31 Musk B* (6,7-[4-oxacyloheptano]-1,1,4,4-tetramethyl-tetralin)		International Flavors & Fragrances The Netherlands	pure
32 Musk A* (2-oxa-4,5,5 8,8 penta methyl 1,1,2,3,4,5,6 7,8-octohydro-anthracene)		International Flavors & Fragrances The Netherlands	pure
33 Musk T* (1,1,6-trimethyl 7-isopropyl 5 acetyl tetralin)		International Flavors & Fragrances The Netherlands	pure

marked with an asterisk these substances were chosen because of their resemblance to other chemicals within the particular groups and because they could be obtained in a very pure state The substances which were not especially purified for these experiments were tested for impurities by gaschromatography As seen from Table I most substances had impurities which were less than one per cent Pyrrole was obtained newly prepared and had only a small amount of impurities when tested a year after preparation and usage The sample of furane had evaporated before it could be tested 2 Methyl 2 butanol contained about 7 per cent impurities The sample of 5α androstan 3β-ol contained 10 per cent of another substance most probably androstanone

TABLE II Chi square matrix for the 'camphoraceous' substances

	2	4	5	6
1 p-Dichlorobenzene	0 101	5 973	11 035	3 237
2 d, 1-Camphor		0 542	10 036	5 702
4 2 Methyl 2 butanol			8 471	15 864
5 3-Methyl 2 butanone				13 569
6 2 Methyl 2-propanol				

[illegible]

tions (Kruskal 1964 a b) In many cases the three-dimensional solution was superfluous, in one case it had a higher 'stress than the two-dimensional one The only exceptions were the two arrays of data from the studies with odours of the cross comparison' and the musky' substances One of the one-dimensional solutions had acceptable stress'

This method of analyzing the spatial arrangement of the comparisons between odours is based upon the rank order between the pairs rather than upon actual measurements The distances in one figure are therefore not comparable to the distances in any other figure

Results

[illegible]nits in the frog s olfactory bulb show irregular spontaneous activity Stimulation [illegible] the receptor epithelium with odours most frequently caused an inhibition of their activity and only about 20 per cent of the units were excited by stimulation The relative number of excited units was lower in the present study than in the previous ones (Doving 1964, 1965, 1966), mostly on account of the larger number of units inhibited The relative number of units unaffected was about the same as in the previous studies A total number of 500 units were studied

'Camphoraceous A total of 83 units were studied for the camphoraceous odours Of these units 16 per cent were excited, 73 per cent inhibited, and the rest unaffected by stimulation

The chi-square matrix is shown in Table II The highest chi-square value was obtained for the pair of 2-methyl-2-propanol and 3-methyl-2-butanol The lowest values were found for the 3 pairs p-dichlorobenzene and d,1-camphor, d,1-camphor and 2-methyl-2-butanol, and p-dichlorobenzene and 2-methyl-2-propanol These pairs were the only ones which gave chi-square values indicating independence

The two-dimensional solution for the camphoraceous substances based upon the data in Table II is given in Fig 2 This configuration of the 5 odours has zero stress,

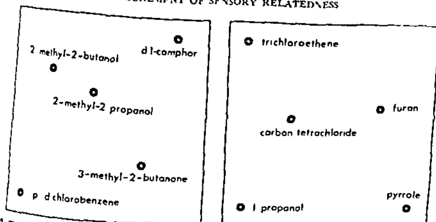


Fig 2 The two dimensional configurations of the odours used in the camphoraceous (left) and ethereal series (right) Zero stress for both solutions

TABLE III Chi-square matrix for the ethereal substances

	9	10	11	12
8 Carbon tetrachloride	17 554	10 668	8 272	18 615
9 Furane		4 762	9 819	5 151
10 1 Propanol			1 211	3 437
11 Pyrrole				1 059
12 Trichloroethene				

compared with 19 per cent stress for the one dimensional solution As seen from the figure the alcohols and ketone were placed in the center of the configuration with p-dichlorobenzene and camphor at each side The monotonicity of the function relating the data to interpoint distances was achieved by equalizing five of the values Whether this is inherent in the data or is a result of the method of analysis is not clear

Ethereal The substances used for representing Amoore s ethereal group excited in average 20 per cent and inhibited about 70 per cent of the 83 units studied Trichloroethene excited about 25 per cent of the bulbar neurones All the substances except propanol evoked unusual electro olfactograms with initial positive deflections (cf, Byzov and Flerova 1964 Gesteland *et al* 1965) most probably due to their deleterious and narcotic effects upon the olfactory cilia These effects made it difficult to estimate the strength of each stimulation and hence to judge the responses of the bulbar units at the same stimulating efficiency

The chi square values were generally higher than in the camphoraceous group (see Table III) Of the 10 pairs seven gave chi-square values indicating that the odours were significantly associated at a 5 per cent level Only the pairs between propanol, pyrrole and trichloroethene gave chi square values indicating independe

TABLE IV Chi square matrix for the floral substances

	14	15	16	17
13 Acetophenone	6 291	0 005	0 231	10 418
14 Anisol		0 441	4 671	0 234
15 Benzophenone			0 176	0 18?
16 Geraniol				0 342
17 Salicylaldehyde				

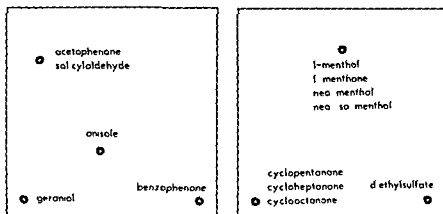


Fig 3 The two-dimensional configurations of the odours used in the floral (left) and minty series (right) Zero stress for both solutions

The lowest value was found for the pair pyrrole trichloroethene The structure in the data of Table III is illustrated in Fig 2 This configuration has zero stress

Floral The substances selected from Amoore s floral group excited an average of 15 per cent of the bulbar units and inhibited about 77 per cent

The chi square values were much lower than in the series mentioned above (see Table IV) Significantly high chi square values were found only for the pairs acetophenone and anisole acetophenone and salicylaldehyde and anisole and geraniol

The two-dimensional solution of the data with floral substances is given in Fig 3 The points representing acetophenone and salicylaldehyde fell together This two-dimensional solution also had zero stress

'*Minty*' A relatively larger proportion (83 per cent) of the 91 bulbar units was inhibited by the substances representing Amoore s *pepperminty group* than by the substances from other series

As seen from Table V the l menthol and menthol derivatives made pairs with high chi square values seemingly constituting a homogenous group of odours A similar group was made up by the cycloketones between which *high chi square* values were also found Most of the other pairs did not show a significant association

TABLE V Chi-square matrix for the 'minty' substances

	19	20	21	22	23	24	25
18 Cyclopentanone	17 605	7 010	4 175	0 079	4 188	1 535	6 549
19 Cycloheptanone		8 496	0 008	1 784	6 074	6 567	6 865
20 Cyclooctanone			4 945	0 232	2 398	0 184	2 562
21 Diethylsulphate				0 059	0 009	0 164	0 378
22 l Menthol					18 836	24 759	13 575
23 l Menthone						7 261	54 712
24 neo-Menthol							8 891
25 neo-iso-Menthol							

TABLE VI Chi square matrix for the 'musky' substances

	27	28	30	31	32	33
26 5α Androstan 3β-ol	12 229	3 490	3 593	14 399	4 721	11 423
27 Celestolide		14 896	8 333	25 357	9 [illegible]	8 494
28 Musk ketone			4 989	5 980	7 746	13 516
30 Musk I				4 723	15 552	10 556
31 Musk B					7 506	7 386
32 Musk X						6 054
33 Musk T						

The configuration of the eight 'minty' odours in two dimensions is illustrated in Fig 3 This solution had zero stress while the three and one dimensional solutions gave stress factors of 0 5 and 27 3 per cent respectively The coincidences of the menthol derivatives of the cycloketones shows the similarities within these odour groups The three interpoint distances are equal and the distances of diethylsulphate from the other odours are the same as the distance between the menthol and cyclo ketones As in the 'camphoraceous' case the monotonicity of the function relating the chi square values to interpoint distances was achieved by equalizing the distances between the cycloketones and between the menthol derivatives

Musky The proportion of units excited by the musky substances used in this study was about the same as in the previous experimental series The relative number of units inhibited by these odours was however smaller than in other series on ac count of a larger number of units being unaffected by stimulation The receptor potentials evoked by stimulation with these substances had slower time courses and were of relatively smaller amplitudes than those elicited by substances used in the other experiments There might be a relationship between the large number of unaffected units and the relatively small amplitude of the EOG elicited by these musky odours

The chi square values given in Table VI indicate a significant association betwee all odours in this series Androstan 3-ol most frequently gave pairs with l square values thus indicating a slight dissimilarity from the other odou

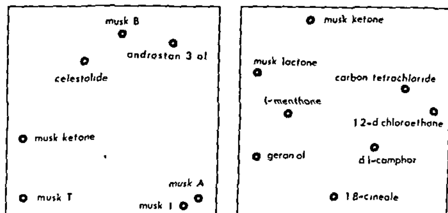


Fig 4 The two-dimensional configurations of the odours used in the 'musky' series (left) and 'cross comparison' (right) Stress 10 9 and 14 6 per cent respectively

TABLE VII [illegible]

	3	7	8	16	23	28	29
2 d, l Camphor	1 514	4 165	1 222	0 657	3 618	0 021	0 790
	0 9	*6 5*	*5 7*	*5 2*	*6 2*	*5 8*	*6 7*
3 1 8 Cineole		1 156	0 035	2 233	0 022	0 090	0 048
		7 3	*6 0*	*4 8*	*6 5*	*5 6*	*7 6*
7 1, 2 Dichloroethane			8 342	0 000	0 097	0 046	0 005
			1 7	*6 7*	*8 0*	*7 0*	*8 1*
8 Carbon tetrachloride				0 970	0 145	0 026	0 512
				5 6	*6 6*	*5 9*	*6 9*
16 Geraniol					2 398	0 870	2 247
					5 5	*2 8*	*4 3*
23 l Menthone						0 194	2 097
						6 6	*7 7*
28 Musk ketone							5 847
							1 9
29 Musk lactone							

The configuration of the seven musky odours given in Fig 4 has 10 9 per cent stress which is 'fair' according to the terminology used by Kruskal (1964 a) The three-dimensional solutions had zero stress while the solution in one dimension had 29 9 per cent stress

'Cross-comparison' The substances used in this series were selected in collaboration with Amoore from his 5 groups to make possible a comparison between the results of his psychophysical studies (Amoore and Venstrom 1966, Amoore 1966, and personal communication 1965) and the results from these electrophysiological experiments

A total of 97 units were tested for these eight odours An av ... of ... these were excited and 75 per cent inhibited

The chi square values for pairs of these substances were ... ne ... pairs in the previous series (Table VII) The pairs 1,2 dich ... ro ... tetrachloride, and 1,2-dichloroethane and camphor gave ... e ... values Most chi square values indicated independence betw ... this series

The probability values showed greater divergence from t ... than in any other experimental series judged by the rank corre ... 0 67 The rank correlation coefficient was, however, significant ... level The underlying structure of the data for the odours of the ... from Table VII is given by the configuration of Fig 4 This solutic ... cent stress which is between 'fair' and 'poor' according to the term ... Kruskal (1964 a) The three-dimensional solutions had 6 3 per cent ... a smoother function relating the chi-square values to interpoint distance ... solutions showed any tendency to group the odours

Discussion

Various psychophysical methods have been used to obtain measurements of the sensory relatedness between odours The easiest and simplest method to perform is seemingly the one based upon quantitative comparison of similarity estimates (Ekman 1954, Engen 1962) Modifications of this principle have been developed by other authors, whose studies have given a large amount of data (Schutz 1964 Wright and Michels 1964, Yoshida 1964, Lange 1966, Amoore and Venstrom 1966) Another method, the so-called 'cross adaptation', is based upon the fact that adaptation to one odour causes fatigue to other odours Since the effect is selective the degree of similarity can be estimated by measuring the extent of threshold variations (Zwaardemaker 1907, Backman 1917, Le Magnen 1948, Cheesman *et al* 1953, 1956, Engen 1963, Koster 1965) The confusion principle was first used in olfactory investigations by Le Magnen in 1950, in studies on odour discrimination in rats The same method has later been applied by Lange (1966) in studies on human subjects Measurements of sensory relatedness based upon electrophysiological data have been obtained by the present author (Doving 1965, 1966) The method is based upon categorizing the effects on the units in the olfactory bulb in this way the data may be subject to enumeration statistics The chi square values and the distribution probability can be calculated for any two pairs of odours in an experimental series The results from the electrophysiological studies are in accordance with psychophysical studies for the odour substances selected from a homologous series (Engen 1962, Doving 1966) The consistency between the results of various psychophysical and electrophysiological studies is statistically significant even for non homologous substances which are very different in odour quality (Amoore 1965, Doving and Lange 1966) The substances used in the 'cross-ad... series provided data for a comparison between the present chi square va...

the method used by Amoore and Venstrom (1966) The results show a significant rank correlation coefficient (0 498) when compared with Amoore s data (Table VII, personal communication 1965) The probability of obtaining such a good correlation by chance alone is less than one per cent The implications of these agreements are further discussed by Doving and Lange (1966)

The 'musky odours seem to be a rather homologous group with significantly high chi square values between all pairs The rather strong relationship between the musky odours indicated by the present findings is interesting in relation to the studies of Guillot and Le Magnen Le Magnen (1948) showed that adaptation to a macrocyclic musk (musk lactone) did not influence the threshold to a keto musk Consistent findings on humans with partial anosmia were reported by Guillot (1948 a, b) who found subjects with very high thresholds for a few musks but normal thresholds for all other musk tested These authors have concluded that the olfactory epithelium contain different types of receptors sensitive to these individual musks, even though they constitute a homogenous group when judged by subjective standards *As demonstrated by the present findings the chi square values are consistent* with the results of subjective judgements

According to the stereochemical theory of olfaction (Amoore 1962) the spatial arrangement of the atoms in the odorous molecules are the most important factors determining odour quality Amoore has classified a large number of odours in seven groups, 5 of which are determined by the stereochemical properties of the substances For two groups (putrid and pungent) Amoore suggests that the overall charge of e molecule is the important parameter for quality determination The concrete rm of this stereochemical theory makes it especially valuable and subjects it to test (*cf* Amoore and Venstrom 1966 Amoore 1966) In the present study the sensory relatedness of the odours in a series have been estimated The odours were selected from five of Amoore s groups and if the classification is valid the results should be consistent with the theory

A reasonable assumption would be that for all pairs of odours within a certain group the measurements of association should be higher than between odours from different groups Similar assumptions should hold when the odours are plotted in a spatial representation of their relatedness Within the spatial configuration the distances between odours of one particular group should always be smaller than the distances between odours of different groups As seen from the present results these assumptions were fulfilled in some cases but not in others for example between substances of the floral group Neither were substances from different groups always independent as seen from the cross comparison In the camphoraceous ethereal and musky series the chi square values found were comparatively large but some odours gave small *chi square values indicating independence In the minty* series the cycloketones and menthol derivates gave high values while small values were found between odours of these two groups The spatial representation also reflects these results by placing the cycloketones and menthol derivates apart from each other in two points

The method of spatial representation (nonmetric multidime nal scaling devel oped by Shepard (1962 a, b) and Kruskal (1964 a, b) can be lied to any data of relatedness such as, for example, the present chi-square o pr ximity values h only requirement is that the function relating the experim ntal d ta to interp distances should be monotone In some cases in this stud the mo otonicit achieved by equalizing a large number of data to the same di ance I oen tal data are represented by the interpoint distances, and the goo ness of f ver the 'stress' (Kruskal 1964 a)

This method of representation is obviously a powerful tool for a lysis of the n lying structure of the data A solution in two or three dimensions or a large numbe of odours will give chemists a clue to the important molecular p ra meters in the determination of odour qualities

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The Effect of Haloperidol and Chlorpromazine on the Amine Levels of Central Monoamine Neurons

By

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Haloperidol and chlorpromazine produce central pharmacological effects similar to those seen after treatment with reserpine Unlike the latter drug, however they have small or no acute effects on the monoamine levels in the brain Nevertheless haloperidol and chlorpromazine have been found to influence the monoamine metabolism by enhancing the accumulation of basic as well as acid catecholamine (CA) metabolites, possibly as a consequence of a blockade of postsynaptic CA receptors (Carlsson and Lindqvist 1963, Andén, Roos and Werdinius 1964) After reserpine treatment the amine levels of certain CA cell groups of the lower brain stem are markedly increased in the recovery phase following an initial depletion Dahlstrom and Fuxe 1964) All these findings may be explained by a compensatory increase of the activity in the CA neurons due to blockade of the neurohumoral transmission from them Therefore it was thought to be of interest to study the effects of haloperidol and chlorpromazine on the intraneuronal monoamine levels in the brain

Male, Sprague Dawley rats (b wt 150—200 g) have been used Two daily doses of haloperidol (5 and 2 5 mg/kg i p) were administered for 1 2 4 or 8 days Three daily doses of chlorpromazine (each 25 mg/kg i p) were given for 2 3 4 or 8 days The rats were kept in a temperature of + 30° C to avoid the hypothermic action of the drugs Food and water was given by a stomach tube The noradrenaline NA dopamine (DA) and 5 hydroxytryptamine (5 HT) levels of the brains of these rats were evaluated both histochemically (see Hillarp Fuxe and Dahlstrom 1966 and biochemically

In the brains of the rats treated with haloperidol a marked increase in fluorescence intensity was observed in many NA nerve cell bodies in the ventro-lateral reticular formation of the medulla oblongata (group A1 according to Dahlstrom and Fuxe 1964) at all time intervals studied (Fig 1) The cells also appeared somewhat swollen and their processes were more distinct than usual This was sometimes true also for the processes of the DA cell bodies of the mesencephalon The 5 HT cell bodies were unaffected The terminal parts of the central CA and 5 HT neurons however showed no certain changes in their amine levels at the various time intervals studied The same was observed also biochemically in the whole brain for the 2-day treatment (NA untreated 0 38 μg/g, treated 0 46 μg/g, DA untreated 0 51 μg treated 0 50 μg/g 5 HT untreated 0 23 μg/g, treated 0 27 μg g)



Fig 1 Effect of haloperidol on the noradrenaline level of nerve cell bodies in the ventrolateral reticular formation of the medulla oblongata (group A1 according to Dahlstrom and Fuxe 1964) Cross-section (a) Normal rat Weakly fluorescent cells are observed × 120 (b) Rat treated with haloperidol for 2 days (see text) Swollen nerve cells with markedly increased fluorescence intensity are seen × 110

After chlorpromazine treatment no increases of the amine levels were observed in the NA cell bodies in the reticular formation of the medulla oblongata However, the DA cell bodies in the arcuate and anterior periventricular nuclei showed a slightly increased fluorescence intensity The processes of the DA cell bodies of the mesencephalic groups also appeared more distinct than in normal rats at all time intervals studied No changes were observed in the amine levels of the NA, DA and 5 HT nerve terminals The same was shown also in the whole brain biochemically for the 3 day treatment NA 0 36 μg/g DA 0 49 μg g 5 HT 0 27 μg/g)

It is of interest that the CA cell bodies showing a marked increase of the amine levels after haloperidol were the same as those showing an increase after reserpine This supports the view that blockade of the CA transmission causes by a compensatory feed back mechanism an increased activity in the presynaptic neuron This may produce beside an enhanced synthesis of the transmitter in the terminals also an increased formation of amine storage granules and amine synthetizing enzymes in the cell bodies

This study has been supported by grants 14X 502 0? 12X 714 0? from the Swedish State Medical Research Council

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